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OBSERVATIONS UPON THE SIZE OF THE SPLEEN.

By J. BARCROFT and J. G. STEPHENS (*Beit Fellow*).

(*From the Physiological Laboratory, Cambridge.*)

By means of a radiographic technique Barcroft, Harris, Orahovats and Weiss(1) inferred that the spleen in the normal animal at rest was usually much larger than the spleen of the same animal post-mortem, and that the change was caused by the discharge of blood from the spleen pulp. If these observations were correct the alterations in the size of the spleen far exceeded delicate changes which demanded the use of plethysmographic apparatus and should be easily visible to the naked eye. Moreover, as pointed out to one of us, the experiments quoted did not absolutely preclude the possibility that the spleen, when it became longer and broader, also became thinner, although there was no reason to believe that this was the case. They were also criticised in conversation on the ground that effects of extra-abdominal pressure on the spleen had not been excluded. These reasons made it desirable to adopt a technique in which the spleen could be observed directly. Such a technique, however, demanded certain conditions proper to the original method, namely, that the animal should be in normal health and spirits, that it should be free from pain and that it should not be under the influence of an anæsthetic.

At first we carried out a number of experiments in animals in which the spleen, though in the abdomen, could be seen through a celluloid window; in the next series we brought the spleen through the muscular layers, so that when the wound healed the spleen was between the body wall and the window; lastly we dispensed with the window, so that the spleen was situated outside the animal, like the testicle. The last of these procedures proved so much the best that the others will be passed over rather lightly.

Granting that the spleen could be placed in such a position as to render it visible, some sort of judgment must be made of the amount of blood which enters or leaves it under given circumstances. It is possible, if the spleen be under a window, to draw a line above the edge of the spleen, marking out on the window the projection of the spleen on the

surface of the window. This may easily be transferred to paper and the area of this projection can be measured. The area of this projection of the spleen on the window will be called in this paper the "spleen area." In the case of a spleen which by operation has been rendered extra-cutaneous a piece of celluloid can be laid over the spleen and a similar projection made. This process is of course a rough one and is not susceptible of dealing with small changes in the size of the organ. But when such a tracing is made, is it possible to discover a relation, even if only approximate, between arterial variation in the bulk of the spleen and consequent variations in the two-dimensional "spleen area"? Amongst the relevant considerations are the following:

(i) If the actual shape of the spleen did not alter, if the increase was uniform in all directions the volume would be proportional to the cube of the square root of the area.

(ii) If the spleen does not increase in thickness to the extent which it increases in length and breadth, cubing the square root of the area will give an exaggerated idea of the change in volume.

(iii) On the other hand it is evident from looking at the spleen that when it becomes distended its angles disappear and its surfaces become rounded. The effect which such changes in shape can produce may be exemplified by an extreme case. Suppose a regular tetrahedron be viewed from above and that its base has an area A; suppose further that the tetrahedron be magnified till its base has an area $2A$: its volume will have increased in the ratio $\frac{(2A)^{\frac{3}{2}}}{A^{\frac{3}{2}}}$. So that if the original area were 100 and the new area 200, the volume would be respectively as 1000 to 2800 (approximately). But now suppose that the new tetrahedron is turned into a sphere, the surface of which passes through the points of the tetrahedron, the volume of the sphere will be of the order of 18,000, or eighteen times the volume of the original tetrahedron. With an irregular object like the spleen, which can alter not only in size, and in the convexity of its surfaces, but also in the convexity of the whole organ (which in the dog and cat is C-shaped), the problem becomes more difficult still. As it seemed less hopeless to obtain a relation between the "spleen area" and the spleen volume by calibration than by calculation, resort was had to injecting blood into the excised spleen and comparing the weight of the spleen with the "spleen area." Four such experiments were performed, two in the dog and two in the cat. All the splenic vessels were carefully tied except the main splenic vein, into which the injection was made; into this a cannula was placed. The animal was bled and its blood

defibrinated; the spleen was then rapidly taken out, weighed and placed on the surface of the animal (as the extra-cutaneous spleen would be); blood in known quantities was injected with a syringe and was controlled by weighing the spleen at intervals. Tracings were taken and the "spleen area" observed after each injection. The procedure was varied a little in different experiments but it was of the same general character in all except the last. In that the dog injected its own spleen. The vessels were tied except the main artery and vein. A "bulldog" forceps was placed on the artery. Cannulæ were placed in the two convergent branches of the splenic vein close to where they emerge from the spleen. The nerves were destroyed. When the "bulldog" was removed from the artery, the spleen swelled up to a great size (of which more will be said later). The spleen without loss of blood was cut out, weighed, and then the blood allowed to gush out of the cannula in the veins. It was collected in measuring cylinders. The spleen was also weighed and tracings taken. The volume measurements and the weighings agreed on the assumption that the specific gravity of dogs' blood was 106. The result of these experiments was to show that the total weight of the spleen was almost exactly proportional to the square of the "spleen area." In one case the actual change was slightly below that calculated; in the other three it was above, and above to a rather greater extent. On the whole, therefore, if the gain or loss is computed as being proportional to the change in the square of the spleen area, the quantity of blood entering or leaving the spleen is likely rather to be under- than to be over-estimated.

The following example will serve: the weight of the spleen at the end of the experiment was 46 grm. as found by actually weighing the organ. 28 grm. of blood has just been injected (last figure in column 3); it may be presumed that the weight previously was 18 grm. (last figure in column 2 carried back to penultimate in column 4). Comparing the squares of the areas before and after the 28 grm. were added, these areas were found to be in the ratio of 19 to 46, as the weight after the addition of blood was 46 grm. and the computed weight before the addition of the blood was 19 grm. (last figure in column 1). In the case of the last observation but one, the organ was presumed to weigh 18 grm. after a measured quantity of 30 grm. had been withdrawn from it, therefore the weight before the withdrawal was presumed to be 48 grm. The ratio of the square of the surface before the 30 grm. were withdrawn to the square of the surface at the end of the experiment was as 47 : 46 and so on.

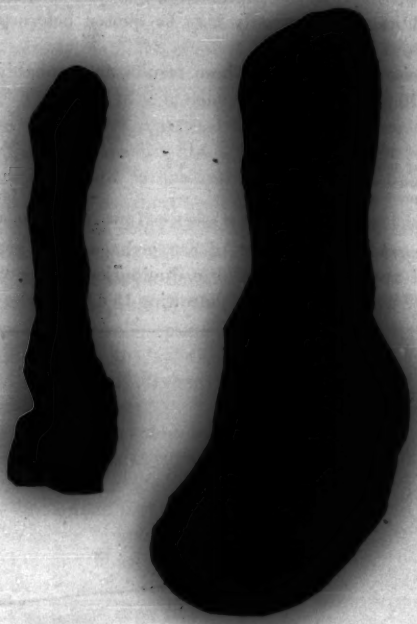
1	2		3		4
Weight of spleen computed as 46 × (surface)^2 ÷ (surface when weight is 46 grm.)^2	Weight of spleen arrived at by adding the sum of the weights : blood injections to 46 grm.		Weight of blood injected in each observation		Weight after injection of blood in column 3
29	24	+	5	=	29
32	29	+	16	=	46
46	45	+	52	=	97
88	97	−	10	=	87
80	87	+	21	=	108
91	108	−	60	=	48
47	48	−	30	=	18
19	18	+	28	=	46

All weights in grams.

The most unsatisfactory feature of the above experiments is that the animals used were not those in which the spleen had been outside the body wall for some days or weeks. An attempt was made to calibrate such a spleen, but the numerous anastomoses with the vessels of the body wall, as well as additional ones in the abdomen, rendered the experiment abortive. In calculating the amount of blood gained or lost by the spleens in our experiments we have assumed that the law which governs their relation of spleen area to spleen volume is the same as that in the spleens which we calibrated and that the change in volume is approximately as the square of the change in area.

The extreme limits of the dimensions of the spleen. The figures given above show a four-fold alteration in the size of the spleen. The question naturally arises: what are the limits within which the spleen is capable of varying? In this connection the following comparison is of interest. Two dogs of approximately the same size (16 and 18 kilos. respectively) were used. The first was killed by bleeding. The spleen weighed 33 grm. The second dog, whose spleen to all appearance was approximately the same size to start with, was the subject of an experiment already described (p. 3) in which the nerves were cut and the veins tied. The spleen attained a weight of 365 grm. or eleven times that of the first animal. Probably even so great a weight falls short of the extreme capability of the spleen, for the region of the solar plexus had been much handled and no doubt the arterial pressure was very low. Fig. 1 shows the relative sizes of these two spleens. Other organs of the body are not susceptible of such changes, or indeed of changes of volume at all comparable. Probably the penis is the only organ which can suffer a tenfold increase in volume. Physiologically speaking there seems no reason why the word erectile should not be applied to the spleen. The above experiment is confirmed by another in which the spleen of a 20 kilo. dog

attained a computed size of 360 grm. under similar circumstances. In the first case (that of the 18 kilo. dog in which the spleen was actually



33 grams. 59 sq. cm. 365 grams. 194 sq. cm.

Fig. 1.

weighed when at its largest) the dog probably contained 1·6–1·8 kilos. of blood in all: of which 340 c.c. at least was in the spleen, which held therefore about one-fifth of the entire blood of the animal.

The technique used for the celluloid window experiments need not be referred to in detail; it was that of Katsch and Borchers(2) as modified by Florey and Carleton(3) and Stephens and Florey(4). We also introduced some modifications which seemed important at the time. The series, however, culminated in our discarding the window and merely observing the spleen when situated outside the skin, as will shortly be

described. The general results of experiments obtained with the celluloid windows will be found in a preliminary communication in the *Lancet*, 13 March, 1926, also in the *Ergebnisse der Physiologie*, XXV, 1926. The following additional points only may be quoted before passing to the extra-cutaneous spleen.

(*a*) *Exercise on treadmill.* These results are probably accurate to within 5 or 10 p.c.

Cat	1	2	3
Area of spleen, rest	15	16	34 sq. cm.
Area of spleen, exercise	8·5	9	21 sq. cm.
Computed weight, rest	—	—	34 grm.
Computed weight, exercise	—	—	14·8 grm.

Cat 3 post-mortem weight of spleen 8·57 grm., area 16 sq. cm.

(*b*) *Hæmorrhage.* The effect of hæmorrhage is to produce an immediate and approximately uniform diminution in the volume of the spleen. Fig. 2 was obtained by withdrawing 15 c.c. samples of blood from

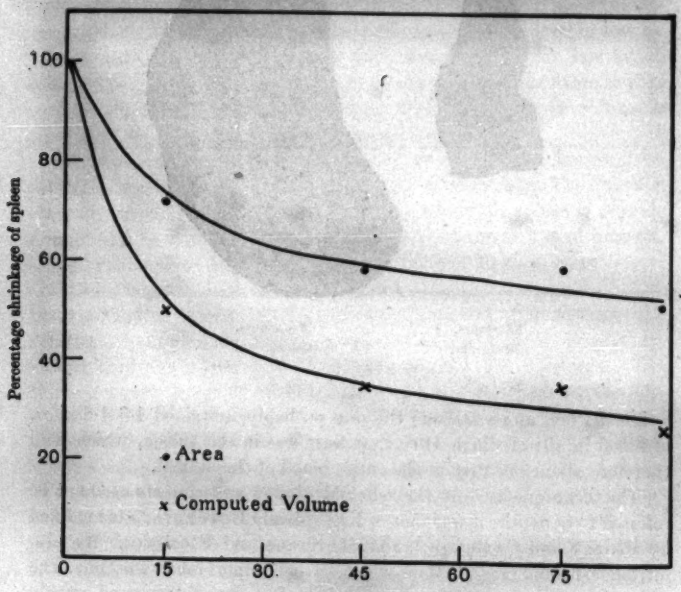


Fig. 2. Effect of hæmorrhage on volume of spleen.

the carotid artery of a cat (under urethane) at ten minute intervals, and represents the percentage diminution in the area and calculated mass of the cat's spleen as the result of such successive hæmorrhages. The result is in close agreement with that of Barcroft, Harris, Orahovats and Weiss(1), namely, that the effect is most marked at the onset of hæmorrhage.

Pneumonia. The onset of pulmonary infection as judged by stethoscopic evidence was always signalised by a gradual shrinkage of the spleen. This progressed steadily for a few days until a reduction in volume of about one-third to one-half had occurred, after which the volume remained almost constant until death.

No reliable conclusions could be drawn from the two cases as to the effects of sepsis at the site of the window, although it seemed that no volume change whatever had occurred.

Administration of oxygen had the effect of brightening the colour of the spleen in pneumonia, in which disease the spleen is dark. Normally administration of oxygen does not affect the colour of the spleen.

Death. The post-mortem spleen irrespective of the mode of death was in all cases much smaller than that during life. Fig. 3 is a photograph of the actual post-mortem spleen placed over the paper template of the same spleen during life one hour previously. The animal in this case was the same one as that employed for Fig. 4, but after it had died of hæmorrhage. This represents a shrinkage to one-third of the volume during life and emphasises the fallacy of visualising the living spleen as being the same size as the dead one.

Fig. 3.

THE EXTRA-CUTANEOUS SPLEEN.

Although experimental animals into which celluloid windows have been inserted exhibit a surprising freedom of movement and are apparently very little embarrassed by the presence of the window, nevertheless great difficulties are encountered in endeavouring to make observations over periods of time greater than about two weeks, principally due to the formation of fibrinous material on the inner surface of the window. These difficulties are partly eliminated by the employment of a

removable window. On the whole, however, the movable window only succeeded in a minority of cases.

We therefore turned to the extra-cutaneous spleen. It was found possible to bring the spleen through a slit in the abdominal wall and to fix it uncovered outside the skin. The spleen thus retains all of its original vascular, nervous and mesenteric attachments and yet grows as a protrusion outside the body of the animal, just as an ear grows as a protrusion outside the head. The only covering for some weeks is the peritoneal investment which undergoes some degree of thickening and fibrosis. Ultimately epithelium grows over the spleen from the edges.

Sepsis does not occur. Lavage with warm eusol every second day or thereabouts, and lubrication with melted vaseline, suffice to keep the spleen for some months as a cutaneous appendage. Observations were thus made on one animal over a period of three months until terminated during a trial demonstration at Stockholm by an automobile accident. Another dog had his spleen outside his skin for eight months.

Detail of operative procedure. The operation has been performed on cats and dogs, but dogs are the more suitable.

A linear incision about four inches long is made through the shaved and sterilised skin of the animal about one and a half inches below the costal margin. This does not coincide with the long axis of the spleen, but it is necessary to draw the spleen as far towards the cephalic end of the dog as possible, in order that the hind limb may not rub against the organ in running and jumping. The extreme mobility of the spleen in most cases permits of this procedure without difficulty.

A shorter incision is then carried through the entire abdominal musculature, the spleen identified, and the length of its mesenteric attachment estimated. The incision through the muscles may now be enlarged so that the resulting slit will accommodate the mesentery of the spleen. The spleen is then drawn through the aperture, and a series of thick cat-gut stitches inserted, firstly through the entire fascial, muscular and peritoneal layers, then through the splenic mesentery, avoiding the blood vessels, next through the peritoneum, muscle and superficial fascia of the opposite side and finally returned and tied. The tying of these stitches calls for some judgment. Too great laxity will permit a hernial protrusion of viscera; too great pressure on the splenic vessels will cause œdema or infarction of the spleen. Moreover, allowance must be made for some degree of post-operative œdema of the muscle. If the stitches are tied fairly loosely, however, the lips of the incision just touching the mesentery, these difficulties will be satisfactorily avoided.

The retracted skin is best fixed by a continuous plain cat-gut suture around the margins of the incision. In this way the spleen exists in a mushroom-like fashion with a median linear attachment to the animal. If a bare area of superficial fascia is allowed to remain underneath the spleen adhesions are inevitable and the borders of the spleen will be obscured by exuberant granulations, as was found to be the case in our first experiment.

Post-operative treatment. The spleen is apparently entirely non-sensitive; the animal will roll on its spleen without concern, and it therefore becomes necessary to cover the organ with a dressing lubricated with vaseline in order to avoid accidental damage. To prevent the animal from licking or biting its spleen, too, some form of covering is necessary except when actual observations upon running, etc., are being conducted, and the dog should be muzzled when not under observation.

The best method of dressing so far devised has been the following. The spleen is first thoroughly irrigated with warm eusol, then anointed with vaseline previously melted and cooled, and then covered with oil-silk sterilised by boiling.

A thick pad of cotton wool is advisable to prevent injury to the spleen, and this may be bandaged in position. Some form of jacket to prevent access of the tongue is necessary until the dog has grown accustomed to the dressing. New dressings are applied every second or third day.

The method of recording and its accuracy. Fig. 4, to be discussed later,

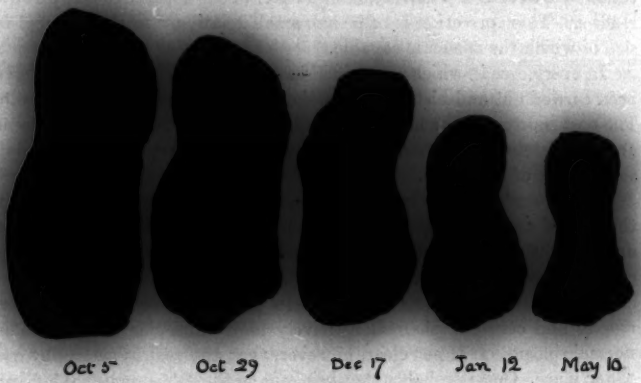


Fig. 4.

illustrates the superficial area of the external surface of the spleen, *i.e.* the projection of the spleen as seen through a piece of celluloid placed over it. In taking the records the area covering the spleen is marked out on the celluloid with a "grease pencil." The celluloid is then removed and the tracing is transferred to a piece of paper either by pressing the paper on to the celluloid, when the mark adheres partly to the paper, or by transmitting light through the celluloid and paper, and marking the paper in ink over the mark on the celluloid. The area is then measured with a planimeter.

In order to show the error as between several successive readings of the same spleen in the resting condition, the following were taken in quick succession.

Three examples are given:

I. 45, 48, 45, 45, 46 sq. cm. dispersion 7 p.c.
II. 44·5, 44, 44, 45 sq. cm. dispersion 3 p.c.
III. 40, 41, 40, 42 sq. cm. dispersion 5 p.c.

It may be taken that any observed alteration of over 10 p.c. represents a real change in the size of the spleen.

There is a personal equation involved in this measurement of the "surface" which the spleen presents. This equation depends upon the amount of pressure put upon the celluloid when in contact with the spleen. As between two persons it is very constant, at least it was so as between Barcroft and Stephens and the readings are all given, in terms of Barcroft, a correction of 14 p.c. being applied to Stephens' readings. This correction is of course small relatively to the changes in size of which the spleen is capable.

In every case in which the animal has been killed, post-mortems have been carried out and histological sections of the spleen have been made. The post-mortems have not shown any hypertrophy of accessory spleens and the histological preparations have revealed little that is abnormal in the interior of the organ.

On the surface there is a thick deposit of white fibrous tissue and in the case of experiments of longest duration this has been overgrown by an invasion with stratified epithelium from the edges. The spleen has ceased strictly speaking to be extra-cutaneous.

The muscular trabeculæ in some cases give the impression of hypertrophy and certainly there is relatively more muscular tissue than in normal spleens. It is not clear, however, that any real hypertrophy has taken place. Quite probably the shrinkage in the spleen may have taken place at the expense of the spleen pulp rather than the muscle and that

the muscle may therefore have got rather "bunched up" and the trabeculæ thickened in that way. There is no real evidence that the spleen contained a greater number of grams of unstriated muscle at the end than at the beginning. For the rest the organ was normal in character.

The size of the spleen. Immediately after the operation the spleen is frequently very large and shrinks considerably in the first few days; when in this condition it must be regarded as hyperæmic, though the cause of this hyperæmia is obscure, there being no evidence of undue pressure on the veins. Nevertheless it is not claimed that the spleen would be so large inside the animal as it is outside during the early days after the operation. This period, however, soon passes off, the spleen acquires a healthy colour and from that point observations on the effect of exercise may be undertaken. After the initial dilatation has passed off the spleen of the resting animal changes little from day to day, but if observed over long intervals of time it is found to be shrinking slowly. The experiment of longest duration so far lasted over eight months, during all of which time the animal appeared to be in perfect health and excellent spirits. Nevertheless the spleen underwent a slow shrinkage, as shown by Fig. 4.

The cause of this gradual diminution in size cannot be definitely stated. As histological preparations have revealed nothing save a covering of scar tissue, one tends to take refuge in the phrase that it is the nature of cicatricial tissue to contract, and there to leave the matter.

Yet there are some other facts which may not be devoid of importance. Not all spleens shrink at the same rate. All dogs which have undergone the operation acquire a reduced hæmoglobin value in their blood. The question arises, is there any relation between the degree of anæmia and the rate of shrinkage of the spleen? These experiments are still being carried out, but the facts may be stated graphically (Fig. 5). They are all taken from observations made during the first six weeks or so after the operation. Ultimately any relation, apparent or real, would probably disappear, because at the end of, say, two months or less the anæmia would probably tend to pass off, but the spleen does not enlarge, if only because it is encapsulated by a dense connective tissue covering.

At the present juncture one must guard against the assumption that because two phenomena can be plotted on the same piece of paper and a curve produced, there is any real causal relation between them. All that the figure shows is that simultaneously two progressive phenomena are taking place, the anæmia and the shrinkage; whether or not they are related can only be decided by experiment, *i.e.* by discovering whether

by imposing one factor on the animal, the other factor necessarily follows.

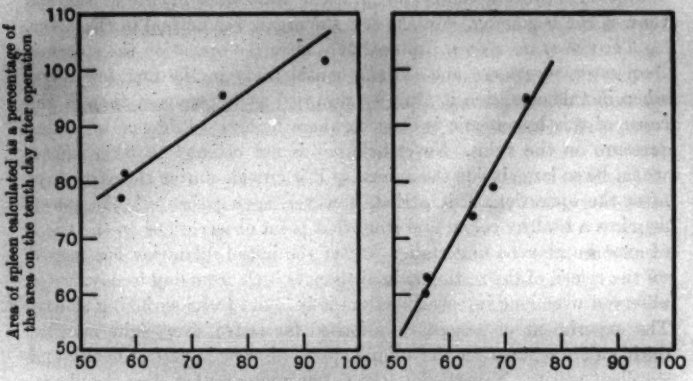


Fig. 5.

This much, however, can be said, namely, that the animals which have become anæmic most rapidly are those in which the spleen has shrunk most rapidly. That merely has been our experience gleaned from the animals which drew our attention to the phenomenon. The subject now demands, and is receiving, systematic work, to be reported on a subsequent occasion.

Relation of area to volume. Within the limits shown above, the size of the resting spleen over short ranges of time is remarkably constant. The result shown earlier in this paper, namely, that the change in volume varies almost exactly as the square of the spleen area applies to the extra-cutaneous spleen; at all events, the changes in volume of the extra-cutaneous spleen are not exaggerated if computed as being proportional to the difference in the square of the areas. Some caution must be used if the changes considered involve long periods of time.

Exercise.

Three forms of exercise have been used:

(1) Running on the level.
(2) Swimming.
(3) Running on the staircase.

The first dog of the present series, "Pongo," was a small Scottish terrier who was easily trained to run about the laboratory grounds after a bicycle, being full of spirit. Our greatest difficulty with her was to get her in a sufficiently quiet mood ever to give us a tracing of what we could regard as a spleen with the animal at rest.

A course of 2000 metres was marked out; the usual time taken for her to run over the course was six minutes. Naturally these figures are very rough, because she did not keep precisely to the course and the time was subject to slight variations.

Inspection of the spleen was even more striking than measurement. It was evident that the shrinkage in thickness was not less than that in length and breadth, and that the spleen which at rest had been deep red became quite pale when discharged. Taking the weights above for what they are worth, the greatest amount of blood which the spleen discharged on exercise was 74 grm. This dog was about four kilos. in weight; its blood volume may therefore have been about 400 c.c. The spleen then discharged 18·5 p.c. of the volume of the blood. There is reason, however (partly on the basis of Cruickshank's, and of Scheunert and Kryzwanek's[13] work, and partly on the basis of work shortly to be published), to believe that the corpuscle value of the blood discharged from the spleen is greater than that in the general circulation. At times the disparity is about 3 : 2 but on the average about 6 : 5. Using the latter figure 74 c.c. of spleen blood would amount to about 86 c.c. of ordinary blood. This calculation would show that about one-fifth of the corpuscles in the body can be held in the spleen and expelled into the blood during exercise.

Effect of running.

Exp.	Date from operation (days)	Distance run (metres)	Computed weight of spleen (grm.)		
			Before	After	Difference
I.	28	500	60	31	29
II.	33	300	66	34	32
III.	32	2000	104	30	64
IV.	34	2000	68	40	28
V.	59	2000	42	12	30
VI.	77	2000	35	16	19

Probable blood volume = 400 c.c.

One suggestive point in the above table is shown by a comparison of experiments II and IV. The sizes of these were much the same at the commencement of the exercise and the same was true of the end, so that there was an equal diminution in size, and that between roughly the same limits; yet the amount of exercise taken in the two experiments

was very different. In experiment II the animal ran 300 metres, in experiment IV 2000 metres. The point is noted here, it will be considered later on.

Not every dog takes kindly to running after a bicycle, and one which did not was the next on which we operated. It was necessary to find some form of exercise for her about which she had no option. Having a large tank in the laboratory animal house we turned it into a swimming bath. Its depth was such that when full she could not touch the bottom. Warm water was laid on so as to preclude the possibility of change in the size of the spleen being due to contact with cold water. Also the spleen was supported, which appeared at the time to be an important point. We thought that possibly the act of galloping might cause an irritation to the unsupported organ to which it might respond by contraction. To a strong swimmer the amount of exercise involved in paddling about in a swimming bath appears to be very trivial, but it can easily be increased by tying a suitable weight to the dog. Most of the experiments were carried out at between 34° C. and 37° C., but no great difference appeared in the result even if the water was not heated.

The following table gives some data for the dog which yielded the results on running tabulated above. Some data are given also for another dog which show that the changes in size of the first were not exceptional.

Effect of swimming.

	Date from operation (days)	Temp. of water °C.	Duration of swim (min.)	Computed weight of spleen (grm.)		
				Before	After	Difference
"Pongo"	28	15	3	68	38	30
	31	37	3	74	38	36
	36	36	3	48	24	24
	60	34	6	38	23	15
"Trixie"	10	34	6	152	88	64
	—	34	6	99	49	50
	18	34	10	93	49	44
	25	34	10	61	40	21

Here again the duration of the exercise—as between three and six minutes—did not seem to have any marked effect.

On the other hand the mere fact of being in the bath had little effect upon the spleen because that organ did not shrink, or it only shrank slightly if the water was shallow enough to allow of the dog touching the bottom with her feet.

Effect of swimming.

No.	Days after operation	Time (min.)	Computed weight of spleen (grm.) Before	After	Difference
			Back feet touching bottom		
1	22	4	63	63	0
2	—	4	50	40	10
3*	—	7	50	34	16
			Swimming		
4	28	3	68	38	30
5	36	3	48	24	24

* Water deeper in 3 than in 2.

Staircase experiments. The best subject which we have had for research is a retriever bitch of 14 kilos. The particular advantage which she has over any dog of the terrier type is that she will remain perfectly quiet when told to do so. Thus she will lie on the table with but little attention and, once she understood what to do, would run up or down the laboratory stairs without wishing to wander off into the passages. The total height of the staircase is 14·2 metres, there are 89 steps.

The changes shown in the spleen of this dog after a run upstairs, which took only a very short time, were even more conspicuous than those of the other two animals which we have described. At the bottom of the stairs her spleen was like a somewhat flattened sausage, and was red or purplish in colour according to circumstances. At the top it was pale and flat—almost concave—so that the tracings only give a partial idea of the weight of evidence which shows the extent to which the spleen discharged its contents.

Dog	Day after operation	Exercise	Dimensions of spleen Before: Area of tracing	Before: Computed weight	After: Area of tracing	After: Computed weight
N	35	Once up staircase	42	102	34	68
	40	Once up staircase	41	98	30·4	54
	47	Once up staircase	41	98	29	42
	43	Once down and up	36	74	20	23
	19	Once down and up	44	104	36	76

The computed loss of blood on these occasions was very nearly the same, namely 22, 27, 30 and 31 grm. respectively.

Only three experiments are given above; in point of fact the routine was gone through many times, the alteration to the eye being sufficient to make the demonstration a most telling one. It is not possible to make

any calculation of value as to the relation of the amount of blood expelled to the total blood volume, because on the thirteenth day after the operation the dog removed the dressing in one place and bit off a large portion of the spleen, which appears to be quite insensitive either to tactile or painful stimuli.

At the end of eight months, the spleen, though small, showed the same proportionate contraction as earlier, namely to about two-thirds its area, or about one-half its weight, as running up the stairs.

It shrank also on the animal's being bled to death to about the same, or to a somewhat greater extent.

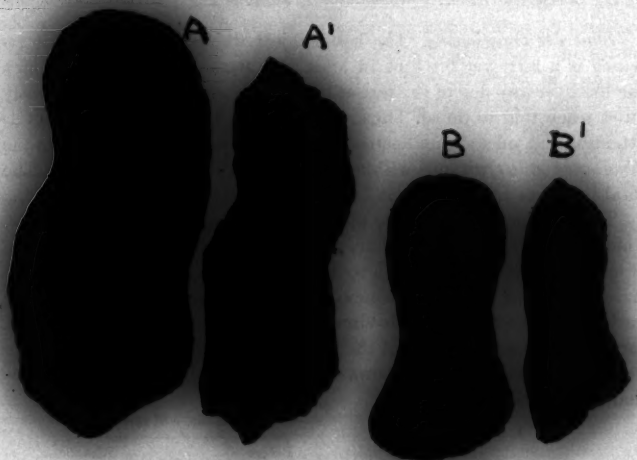


Fig. 6. *A* rest. *A'* after ascent of staircase, 29 October, 1926.
B rest. *B'* after ascent of staircase, 8 May, 1927.

Relation between the amount of exercise and the amount of contraction. On the whole the general rule holds that the greater the amount of exercise, the greater the amount of contraction; thus in the foregoing tables, a longer swimming effort or a longer running course usually produced a greater degree of evacuation of the spleen. The striking point, however, is that the response to very mild exercise is proportionately greater than to severe exercise.

Thus to casual inspection the degree of contraction and pallor of the

spleen caused by running up the staircase, involving roughly 200 kilogram-metres of work against gravity differed little from the corresponding effects of running down the staircase in the case of one dog (Nigger); in the case of another (Pongo) a run of 70 metres from one end of the laboratory passage and back caused a computed shrinkage of volume of from 74 to 38 grm. whilst a run of 200 metres after a bicycle only produced shrinkage of from 74 to 28 grm.

The conclusion seems to be that in exercise as in hæmorrhage the most marked effect relatively is in the initial stage, that expulsion of blood into the circulation is far from being a final effort on the part of the organism to adjust the volume of the circulating blood to that of the bed in which the blood circulates; it is in fact a fine adjustment and it can be done without, like most fine adjustments.

Mechanism of the splenic contraction with exercise. Hargis and Mann(6), in a most convincing research, demonstrated the sensitiveness of the spleen to nervous influences in the unanæsthetised animal. de Boer and Carrol(7) showed that the contraction of the spleen which was induced by administration of carbon monoxide, was operated from the central nervous system; in an animal to which the spleen was attached by the nerves only, it contracted when the animal inhaled the gas: the spleen, however, received its blood from a perfusion circuit and introduction of CO into the blood did not lead to contraction of the organ. The contraction caused by exercise is also of nervous origin, or at least principally so. The alteration in size is not marked when the nervous connections are severed. A striking demonstration is furnished by severing all the nerves which run along those branches of the splenic artery that supply the ventral and larger portion of the organ, whilst the nerves which supply the dorsal portion are left intact. This method of demonstration was suggested to us by Dr Anrep. We are indebted to Dr Lim(14) also for sending an account of a very similar experiment performed by him. Our experience has been that on exercise the denervated portion suffers a very slight decrease in area with a certain increase in flabbiness, whilst the intact portion contracts, as does the ordinary spleen, to about two-thirds of its area, during the ordinary staircase experiment. Such a contraction would mean halving the volume.

Fig. 7 shows the effect of one ascent of the Laboratory staircase by a dog in which the top portion of the spleen was innervated normally and the lower portion denervated (as far as is known, for the dog being still alive, no post-mortem has been done on the spleen). The thin horizontal

line between *A* and *B* separates the innervated and denervated areas, so far as can be seen. The following are the areas of the two before and after exercise:

	A	*B*	Total
Before exercise	12·5	21·5	34 sq. cm.
	8·5	20·5	29

It appears likely that there is, either due to a lowering of the pressure in the portal vein, or for some other reason, a certain small loss of blood by even the denervated spleen, but that the cause of the principal expulsion of blood in the normal organ is active contraction of the spleen due to nervous excitation. Exactly to what extent this nervous mechanism plays on the splenic musculature as opposed to the musculature of the splenic vessels is uncertain and possibly differs in different spleens: the spleens of some animals contain much more muscle than those of others. The hard consistency of the contracted spleen suggests, however, that the splenic musculature is heavily involved, though that does not exclude the vaso-motor mechanism from participating. Indeed it may be that there is no rigid distinction between the one and the other; the whole musculature of the spleen may with some reason be regarded as of a piece with that of the splenic vessels (the splenic veins are richly endowed with muscle). The principal evidence against such a view is based on the action of drugs, pituitrin acting, according to de Boer and Carroll(8), on the vasomotor mechanism and not on the spleen muscle. Such evidence does not seem to suggest more than that some differentiation has taken place. It does not seem to prove any fundamental disparity in origin. It has been shown by several authors (Bayliss(9), Masuda(10) and Hoet(11)) that the spleen dilates on stimulation of the central end of the depressor; here again there is no evidence as to how far the musculature of the organ as opposed to that of its vessels was involved.

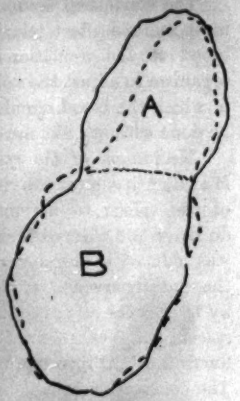


Fig. 7. Rest ——. After ascent of staircase - - - - -.

The speculation that the splenic musculature may be typical of the vasomotor musculature of the visceral area generally leads to the general consideration of the responses of that area to anoxæmic conditions and

to the inducement of oxygen want by exercise. That field is too wide to justify exploration on the basis of our present experiments.

So far as the spleen is concerned the investigation of exercise was undertaken because exercise presented a particular form of oxygen want, but a form to meet which the body has become very highly specialised; it is not surprising therefore to find that, as in the cases of the pulse and the respiration, the nervous control of the spleen has learned to anticipate the actual exercise and the spleen will respond to the type of mental agitation which if pushed would culminate in physical violence. Two examples can be furnished: (1) The first is from the dog mentioned above, one half of the spleen of which was denervated. This animal became greatly attached to its keeper whom it always was anxious to follow. If when it was lying on the table in the hands of another attendant the keeper left the room, the denervated portion of the spleen shrank so that the picture approached but did not quite attain to that of the dotted line in Fig. 7. On being released it bounded off the table after the keeper. This reaction could be depended upon absolutely to take place. (2) A dog of an extremely jealous disposition. While she lay on the table her spleen would normally remain of constant area, but if her kennel companion were fondled by the keeper in her presence, her spleen would become smaller and grow pale.

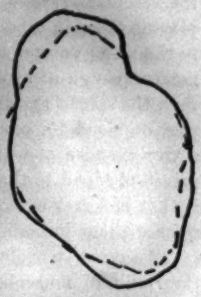


Fig. 8. Size at rest ———. Excited by fondling a rival - - - - - -.

The change in size is shown in Fig. 8.

THE SIGNIFICANCE OF THE SPLENIC CONTRACTION DURING EXERCISE.

(*a*) *In relation to the vascular bed.* The question cannot fail to be asked: to what extent does it matter whether either the blood volume or the volume of circulating hæmoglobin receives a considerable augmentation when exercise is taken? The discussion is a difficult one owing to our almost complete incompetence to express the importance of the factors involved in quantitative terms. We know for instance that if exercise be taken the "vascular bed" in the region exercised increases in volume. If it is a matter of importance (and within unknown limits it must be) that the volume of blood should bear some relation to the capacity of the bed in which it lies, then there would be some merit in having a reservoir

of blood outside the circulation which could adjust the volume of circulating blood to the capactiy of the vascular bed. It could then enable the vascular areas in the active organs to open up without unduly employing those in other places. It seems probable that a 20 p.c. variation in the blood volume would go a great way in carrying out these adjustments, though of course it is known that in active exercise other areas than the spleen are drawn upon, *e.g.* the kidney. The body does in fact provide blood for one organ at the expense of another. If in the most active exercise of which an animal is capable, the kidney may be deprived of blood to the extent of being actually injured, how desirable is it that in lesser exercise some store should exist which may be drawn upon without imposing a tax on such important an organ as the kidney.

In a great struggle it is desirable that the animal should overcome as the result of muscular strength, even though a transient albuminuria accompanies the victory. The muscles must have the last ounce of blood which they can get, but on lesser occasions the vital organs may count themselves fortunate that before their blood supply need be drawn upon the spleen can produce a considerable quantity.

(*b*) *In relation to the minute volume of circulating blood.* In the heart lung preparation, the simple way of increasing the output of the heart is to take a jug full of blood and pour the contents into the open venous reservoir. But with a closed circulation blood can only be put into the heart in increased quantity at the expense of what is circulating and therefore prejudices the position for the period of time immediately following.

The spleen pulp, however, being unlike other organs outside the circulation, is, in effect, such a jug. The blood expelled along the splenic vein when the spleen contracts is blood added to the circulating fluid. That may be admitted, but it does not furnish an answer either way as to whether such an action on the part of the spleen is on a scale sufficient appreciably to influence the circulation rate. Nor can an answer be given in our opinion, for it depends upon factors as yet undetermined; the most that can be done is to state the problem more definitely by a numerical example which involves quantities of possibly a reasonable order.

The following example probably flatters the power of the spleen.

Suppose (1) that the blood volume of a man is five litres, (2) that he can expel one litre of blood from his spleen, (3) that this can be expelled in 12 seconds, (4) that his minute volume of blood is five litres at rest. In that case one litre of venus blood reaches the heart in 12 seconds. Starting from zero time, suppose the spleen contracts during 12 seconds

and adds a litre of blood, so that two litres of blood reach the heart, one from the general circulation and one from the spleen. The venous inflow to the heart during that time will be doubled, and the arterial outflow during 12 seconds will be doubled. If during that 12 seconds the vascular conditions in the body become so readjusted that the venous inflow during the next 12 seconds (*i.e.* the 13th to the 24th second) becomes doubled because the arterial output is doubled during seconds 1–12, the whole circulation rate will have become doubled until something takes place to alter that condition of affairs. The power of the spleen then depends not merely on the amount of blood which the spleen can contribute relatively to that in the body, but upon the rate at which the blood from the spleen can be thrust into the venous system and also the rate at which a fresh cyclic equilibrium can be established in the circulatory system. These factors are unknown.

Even could the events just set forth take place in the times and on the scale stated, the blood flow would only double. That is a much smaller increase than takes place during active exercise, but a twofold increase forms an appreciable proportion of the whole and if it occurred would bring the splenic contraction within the group of factors which when integrated accounted for the total increase in the circulation rate.

Summary.

1. Methods for placing a celluloid window over the spleen have given fairly satisfactory results for about a fortnight.

2. Much better is the method of making the spleen extra-cutaneous; as such it has been kept under observation for more than eight months. During that time it underwent a gradual diminution in size. Its function remained unimpaired except in so far as functional changes bore a constant proportion to its size.

3. Both methods show that the spleen contracts to about one-half to one-third of its size on exercise and to an even smaller volume on death or severe hæmorrhage.

4. The amount of blood squeezed out during exercise is estimated at its maximum as forming one-fifth of the volume of blood in circulation; the spleen under such circumstances becomes pale.

5. The events described in paragraphs 3 and 4 (above) depend on the integrity of the nervous supply of the spleen.

6. Psychological processes calculated to culminate in violence may also cause the spleen to become pale and to contract somewhat, thus anticipating actual exercise. In any case the contraction of the spleen is

an early event in both hæmorrhage and exercise, rather than a last resort for the acquisition of blood.

7. The significance of the splenic contraction on exercise is discussed (*a*) with regard to the relation of the blood volume to the vascular bed, (*b*) with regard to augmentation of the minute volume of circulating blood.

The expenses of this research up to 31 December, 1926, were borne largely by the Hæmoglobin Committee of the Medical Research Council; since that date by a grant from the Royal Society. To these we would wish to express our thanks.

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THE BLOOD IN THE SPLEEN PULP.

By J. BARCROFT, F.R.S. and L. T. POOLE, *Major* R.A.M.C.

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The investigations described below were undertaken in order to supply, if possible, independent evidence of the observations made by Cruickshank(1) and Scheunert and Krzywanek(2) that the blood which comes from the spleen during its contraction contains a greater quantity of hæmoglobin than that in the general circulation. The extra-cutaneous(3) spleen preparation described in the previous paper serves excellently for this purpose. If the spleen be stabbed with a needle, or better with a hypodermic needle, blood as a rule issues. This blood is not necessarily pure blood from the spleen pulp; the needle of course penetrates the capsule and may enter trabeculæ, or pierce vessels of considerable size. Nevertheless in so far as it differs from the blood of the general circulation, it will probably reflect the composition of the contents of the spleen pulp. Two methods have been adopted for testing the blood, the hæmatocrit and the Haldane-Gowers hæmoglobinometer. Controls were taken from the ear vein, and, as is emphasised later, from the capsule of the spleen.

Experiments were made (1) with the hæmatocrit, (2) with the hæmoglobinometer.

(1) For the determination of the corpuscle volume blood was collected in the following way. From a piece of sheet rubber about 1 mm. thick a portion was cut about 2 cm. by 1. In the middle of this a hole was punched of about 3 mm. diameter. A little vaseline was placed on the bottom of this small piece of rubber and it was laid on the spleen. A stab was made in the middle of the hole with a needle; the rubber formed a small cell which collected the blood, a pair of hæmatocrit tubes was filled with it and was rotated for ten minutes, after which time the centrifugalisation reached a state of finality. A trace of dry heparine was put on the needle with quite satisfactory results. The spleen is quite insensitive. The dog apparently was usually not conscious of the prick.

The first pair of readings was taken from the ear vein. Readings from there always agree well with one another and with readings taken from an artery. Thus, taking duplicates on different occasions:

Hæmatocrit readings ("Nigger").

Ear vein	(1)	36	37	33	38	34
	(2)	37	35	33	36	34
Artery	(1)	—	—	—	—	33
	(2)	—	—	—	—	33

It may be taken, therefore, that a disparity of more than 3 p.c. in the readings either of duplicates, or as between one vessel and another, has some real meaning.

To turn to the readings from the spleen; the first pair was taken usually immediately after exercise; the spleen was pale and shrunken; the duplicate readings agreed well; it is usual for the blood from the first puncture to have approximately the same hæmoglobin content as that of the ear vein. Once they were markedly below those of the ear vein. This proved a very unusual occurrence; it may be that we went into a lymphatic or some serous cavity. And after all in view of the fact, which will be shown, that the corpuscles become more concentrated in the spleen than in the blood, there must be some situation in which the reverse is true, *i.e.* in which the fluid draining away from the corpuscles is capable of being tapped.

We pass now to the subsequent three punctures. In these readings from the spleen the duplicates differed greatly. In each pair one reading was much above that of the ear vein, the other was either approximately the same or a few per cent. higher. Of such a pair of readings the first taken was always the highest. It occurred to us that the cause of the great disparity might be as follows: that both samples of blood were a mixture of fluid coming from the pulp and from the capsule, that in the first sample the preponderance of blood might have been from the pulp, but that this source quickly dried up, leaving the later hæmorrhage from the superficial area. Or it might have been the other way round, the more concentrated blood from the capsule and the less concentrated from the pulp. It is quite easy to obtain a sample of blood from the capsule by making a puncture which does not go below that investiture. The result is always a sample not richer in hæmoglobin than the blood from the ear vein.

Thus

Hæmoglobin value (H.-G. hæmoglobinometer).

	Exp.		
	(*a*)	(*b*)	(*c*)
Ear vein	67	73	71 p.c.
Capsule	64	75	71
	63	71	72

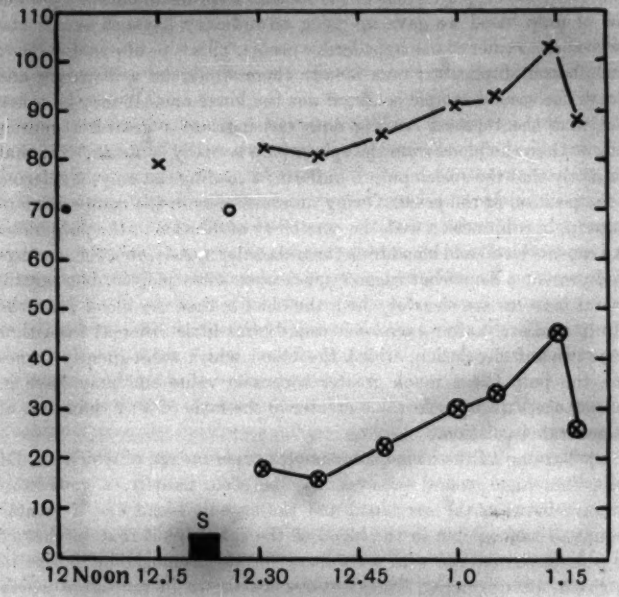
The above figures show that the blood from the ear vein and the capsule agree very well in hæmoglobin content and they cannot differ greatly in corpuscle content. Presumably then the high readings are those which come from the deeper regions of the spleen. In order to obtain a greater flow of pulp blood we gave up using an ordinary bayonet needle and resorted to a rather coarse hypodermic needle. This type of puncture gave much better duplicates; occasionally there would be a disparity and always the second sample collected was the lower one. It may be taken then that the topmost reading does not indicate a greater corpuscle content than the blood from the spleen pulp actually contains. Not that it is likely that the spleen pulp is uniform; a reading can only be referred to the position of the prick. Owing to unevenness in the composition of the pulp, in conjunction with the possibility of dilution of the pulp blood with capsule blood and blood from the trabecular muscle, etc., the readings must present a somewhat rugged appearance when plotted, but certain general features are clear of which the chief is that the blood from the pulp immediately after exercise is usually but little different from that of the general circulation, whilst the blood which subsequently comes from the pulp has a much greater corpuscle value and may have in extreme cases a corpuscle value greater in the ratio of 3 : 2 than that of the general circulation.

(2) Turning to the hæmoglobinometer experiments, of which we did three, the same general features may be seen, namely, a good concordance between the ear blood and the capsule blood and a greater amount of hæmoglobin in the blood of the spleen pulp than in that of the general circulation, with a tendency for the hæmoglobin to rise in the spleen after exercise. These features are shown in the figure which is a fair example of four carried out on the same dog, about six months after the spleen had been brought outside the body.

The fact having been established that the corpuscles become concentrated in the spleen pulp, the question must arise: By what mechanism is the concentration accomplished?

The concentration appears to depend upon the integrating of the nervous supply of the organ. The matter was tested in a dog, the upper portion (*i.e.* that nearest the backbone) of whose spleen was not denervated while the lower portion was. The method of denervation consisted in stripping the nervous elements from the vessels supplying the spleen and cutting away all adventitious material. The only connection between the lower two-thirds of the spleen and the rest of the body consisted in the denervated vessels. The upper portion of the spleen was innervated

normally. The denervation was effected at the same time as the spleen was made extra-cutaneous; that was about six weeks before the experiments here recounted took place.



Readings on hæmoglobinometer (ordinate).

Upper curve: blood from ● ear, ○ capsule, × spleen pulp.

Lower curve: percentage excess of hæmoglobin in spleen pulp as compared with general circulation.

Abscissa: time. Signal (S) = staircase exercise.

The innervated portion of the spleen reacted just as any normal spleen would have done. After exercise the haemoglobin value increased, and attained to a value, as a maximum, of about 50 p.c. higher than that of the blood in the normal circulation; thus on one occasion that of the general circulation gave a reading of 72 on the Haldane-Gowers hæmoglobinometer, whilst that of the spleen pulp was 106.

The denervated portion of the spleen never yielded blood which showed more than the most trifling sign of concentration. In these

experiments further precautions were observed in the collection of the blood from the spleen pulp. The denervated part was disinclined to bleed on an ordinary puncture. The blood was then collected, both from the innervated and denervated portions, in a glass pipette fitted at one end with a rubber teat and drawn at the other into what amounted to a hypodermic needle of glass, *i.e.* into a glass capillary stout enough to be thrust through the capsule. Heparine (powder) was used to prevent coagulation. Even after the above mode of collection was employed, however concentrated the blood found in the pipette, that which flowed from the site of the puncture subsequently was of the same hæmoglobin value as the blood of the general circulation.

Here also recurred a phenomenon to which attention has already been directed, namely, that the high hæmoglobin value is obtained only from the first blood withdrawn from a puncture. Blood which flows from it subsequently is of the hæmoglobin value of the general circulation. This seemed so definite as to make us doubt the adequacy of the explanation already given, namely that such blood merely comes from the capsule. It seemed possible that the damage to the finer structure of the spleen pulp caused by the puncture had been such as to impair the machinery necessary for concentration in the immediate vicinity. The efficiency of the mechanism would seem to depend upon its intactness.

The following figures illustrate the point:

Hæmoglobin readings.

[Gowers hæmoglobinometer with Haldane's CO standard.]

Ear vein	72		72		
Innervated portion of spleen	87	74	105	70	68
Denervated portion of spleen	71	69	72		

The figures under the brackets are successive samples from the same puncture.

The fact of innervation seems most likely to influence the concentration of the blood through some muscular mechanism. Such a function might be connected with the rhythmic contractions of the organ; at least those contractions await some intelligible rôle. They are usually most considerable just after the spleen has been in contraction. They then gradually damp off. There is reason to believe that blood will not pass backwards from the spleen pulp into the artery, at all events it is said not to be possible to inject the artery from the vein, the pulp merely becomes distended. If then at each contraction lymph were squeezed out (as it is from a lymphatic gland (Florey(4)) when the muscle of the gland

contracts) and if at each relaxation some fresh blood entered the pulp from the artery, a mechanism would exist by which the corpuscles could be concentrated in the pulp. It is fair, however, to point out a criticism made to us by Dr Duncan Scott, namely, that we had probably destroyed the lymphatics as well as the nerves.

This is perhaps a suitable place to say a few words in discussion of some recent research. Scheunert and Binet and their respective co-workers have carried out researches in which they have found a considerable increase in the number of red blood corpuscles per cubic millimetre of circulating blood during conditions of exercise, asphyxia, etc., in the majority of which the polycythæmia has taken place in the normal but not in the splenectomised animals. Scheunert and Krzywanek(2), for example, in the horse found that after five minutes' work the red corpuscle count rose from 7·4 to 9·3 millions per cubic mm. and the corpuscle volume correspondingly rose from 29 p.c. to 36 p.c. (taking the average of the two jugular veins). This they attribute in part to the abstraction of water from the blood, but not entirely, for the changes in the refractive index of the plasma proteins, though they indicate a loss of water, do not indicate a sufficient loss of water to account for the whole increase in red cells. Nor is the increase in white cells commensurate with the increase in red cells. The question arises to what extent can the contraction of the spleen account for a change in the corpuscle volume of say from 29 to 39 p.c. (*i.e.* of approximately 25 p.c. of the final volume). The weight of the dead spleen was 2·6 kilos.—that after exercise on the analogy of dogs would be about 3 kilos. and before exercise would probably not be more than 9 kilos. on the same analogy. The corpuscles form 29 p.c. of the circulating blood and 61 p.c. of the blood added by the spleen (such is stated by Scheunert and Krzywanek). Let us further assume that the initial volume of blood in the animal was 40 litres.

	Corpuscle volume litres	Plasma volume litres	Total volume litres
Initial blood	11·6	28·4	40
Blood from spleen	3·7	2·3	6
	15·3	30·7	46

The corpuscle volume is now about 34 p.c. instead of 29 p.c.; so far no reabsorption of plasma by the tissues has been considered. The authors judge from the refractive index that such a reabsorption takes place and that the blood may be reduced to its original volume; if so there would be 15·3 litres of corpuscles in 40 litres of blood, which would be 38 p.c. That is something of the order which they noted. Whilst the

calculation is within the bounds of possibility it seems to us unlikely that the spleen has contributed the whole of the corpuscles which form the basis of the increased corpuscle volume; it appears more probable that the blood volume has increased and that some corpuscles have come from places other than the spleen. Any exact correlation between the nervous splenic mechanism which is responsible for the expulsion of the blood from the spleen pulp and the factors which regulate the imbibition of water by active muscle is not known to exist.

Somewhat similar experiments have been carried out by Binet(5) and his pupils, notably on asphyxia and hæmorrhage. Take one of their examples on the dog. The animal was made asphyxial, and with the pedicle of the spleen compressed the corpuscle count rose during asphyxia from 8·2 to 8·6 millions. The pedicle was released and there was a further rise from 8·6 to 9·3 millions. If these figures were applied to a dog with 400 c.c. of blood whose splenic blood contained 33 p.c. more corpuscles than the normal blood, the spleen would be expected to supply 100 c.c. of blood—that in our opinion is too much, at all events it is more than could be expected of an extra-cutaneous spleen. The principal difference between the basis of the above calculation and that made with regard to Scheunert and Krzywanek's experiments is that the latter authors definitely state that the blood in the splenic vein of the horse contains a corpuscle volume 100 p.c. greater than that for the general circulation and for Binet's asphyxial dog we have assumed 33 p.c. There is no evidence from the extra-cutaneous spleen suggesting that the venous blood from the dog's spleen contains anything like twice the volume of red cells found in the general circulation, but of course the extra-cutaneous spleen may not function so perfectly as the organ in its normal situation. Dr Feldberg of Berlin has informed us privately that he has obtained results which confirm the scale of splenic contraction involved in Binet's experiments.

There seems to be no doubt, however, that the spleen does play a considerable rôle in the increment of corpuscles both during asphyxia and during exercise, even though it may not be responsible for the whole phenomenon.

The expenses of this research have been largely borne by a grant from the Royal Society.

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THE ACTION OF VISIBLE LIGHT ON THE HÆMATOPORPHYRIN SENSITISED ORGANS.

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THE biological importance of short waves of light is commonly recognised, but careful investigations have not shown any significant action of visible light on the biological functions of the animal.

It was found, however, that many fluorescent dyes sensitise biological reactions to the action of visible light. The fluorescence of these substances consists of much shorter waves than the waves of light which they are absorbing.

Hausmann(1, 2, 3, 4, 5) showed that hæmatoporphyrin, which is a fluorescent substance, can sensitise not only protozoa and blood cells but also whole organisms of higher animals to the action of light. Mice injected with hæmatoporphyrin become very sensitive to light. The exposure of such animals to the action of the sun causes acute skin inflammation followed by death. Only meso- and homo-porphyrins show any hæmatoporphyrin-like action; other derivatives of hæmoglobin or chlorophyll are without light-sensitising properties(6). Hæmatoporphyrin alone is not changed chemically when exposed to the action of light(7).

Fischer(8) found that the urine and fæces of light-sensitive men such as those suffering from certain skin diseases (variola vacciniforme) contain hæmatoporphyrin-like bodies. Meyer-Betz(7) showed that man can be sensitised to the action of light by injections of hæmatoporphyrin. The symptoms of this artificial light-hypersensitivity were similar to those described in human disease.

Hæmatoporphyrin seems to be a general sensitiser of photo-chemical reaction. Photographic plates can be sensitised to the action of yellow light by means of hæmatoporphyrin solution. Sunlight causes a destruction of fibrinogen in the presence of hæmatoporphyrin solution. The systemic action of visible light on the organs of hæmatoporphyrin-sensitised animals has not been studied previously. The consideration of this problem forms the substance of the present paper.

The hæmatoporphyrin used in the following experiments was prepared according to Willstätter's modification of the Nencki-Zaloski method. A 1 p.c. solution of this substance in a corresponding quantity of sodium hydrate was used for the experiments. The *p*H of this solution varied between 7·9 and 8·2.

The source of light in my experiments was gas-filled electric bulbs either 200, 150 or 50 candle-power.

The action of light on sensitised smooth muscles.

Adler(9) observed that plain muscle contracts when exposed to the action of ultra-violet light. Identical results were obtained on exposing the phloxine or eosine-sensitised muscle to the action of visible light. In my experiments the organs were sensitised by means of hæmatoporphyrin solution.

Rabbit intestine. Visible light of 150 c.p. at 10 cm. distance causes no change in the rhythm or tonus of the isolated rabbit intestine. A solution of 1/10,000 hæmatoporphyrin in Ringer has also no effect on the movements of the intestine. A stronger solution such as 1/1000 causes a decrease of the intestinal automatic movements.

The exposure of a piece of intestine suspended in a 1/50,000 hæmatoporphyrin solution to the action of light, 150 c.p. at 10 cm., causes a marked decrease of automatic movements and an increase of tonus. This intestine when exposed to the action of light for 15 minutes shows

Fig. 1. Isolated rabbit's intestine. Light 150 w. electric bulb, nil 5 c.c. hæmatoporphyrin added to bath to make 1 in 150,000 = nil. The figure shows the subsequent action of the same light.

complete inhibition of movements, and the muscle no longer responds to the action of histamine or barium salts.

The production of intestinal tonus depends on the intensity of the light: very feeble light causes a slow and incomplete contraction. Light of intensity 40 c.p. at 24 cm. has no effect on the hæmatoporphyrin-sensitised intestine. Different coloured lights also exhibit a different action. Red or blue light is without effect, green causes a slight contraction, while yellow rays produce the maximum contraction. The light which passes through 1/500 hæmatoporphyrin solution causes no contraction of sensitised intestine. These experiments indicate that the most active rays are absorbed by hæmatoporphyrin solution. This is in agreement with the results of Fabre and Simmonet(10) obtained with hæmatoporphyrin-sensitised erythrocytes. These experiments require controlling photometrically.

The contraction of the intestine is not dependent on the strength of the hæmatoporphyrin solution: the minimal concentration of hæmatoporphyrin which causes a marked contraction of intestine is 3/500,000. Greater strengths than 1 in 20,000 do not increase the action of the solution and cause no change in the latent period or in the intestinal tonus. Much stronger solutions of this substance are necessary to cause contraction of an intestine when suspended in rabbit's serum.

The intestine absorbs hæmatoporphyrin from the solutions. A piece of intestine which has been left for 15 minutes in 1/5000 hæmatoporphyrin solution and washed for 20 minutes in Ringer is very sensitive to the action of light.

Rabbit, uterus. Rat, uterus. A virgin rabbit's uterus suspended in

Fig. 2. Isolated rabbit's uterus (virgin). Light = 0. Hæmatoporphyrin 1 in 10,000 = 0. Light after hæmatoporphyrin shown in figure. Time = 30 sec.

1/10,000 hæmatoporphyrin solution, on being exposed to the action of light (150 c.p. 10 cm.) contracts gradually. Under similar conditions the contraction of the non-virgin uterus is more rapid.

The isolated uterus of the rat sensitised by the presence of 1/50,000 hæmatoporphyrin in Ringer's solution contracts gradually in the light of 150 c.p. 10 cm. power, and the automatic movements slowly cease.

Fig. 3. Rat's uterus (virgin). Light = 0. Hæmatoporphyrin 1 in 50,000 = 0. The figure shows the subsequent action of light. Time = 30 sec.

The following isolated and hæmatoporphyrin-sensitised tissues are sent into contraction by the action of visible light: spleen, aorta and bronchi of the rabbit; and the aorta, pulmonary artery and vena cava of the sheep.

Frog, stomach and bladder. Visible light (150 c.p. 10 cm.) causes a gradual contraction of the isolated bladder or the stomach of the frog sensitised in a solution of 1/50,000 hæmatoporphyrin. This contraction is much slower and more gradual than the plain muscle of warm-blooded animals. The automatic movements of these organs of the frog soon cease under the influence of light, and a long exposure such as 45 minutes causes death.

The action of light on the organs in situ.

Cat, intestine. The intestinal movements of a cat anæsthetised by chloralose were recorded by the rubber balloon method. Visible light of 150 c.p. at 30 cm. power passing through a glass window into the peritoneal cavity has no effect on the tonus or peristalsis of intestines. Also the intravenous injection of 0·03 grm./kg. of hæmatoporphyrin in the dark causes only a slight decrease of peristalsis. Subsequent exposure of these intestines to the action of light causes a local vaso-constriction associated with a rise of blood-pressure and depression of intestinal

movements. The tonus of the intestine is increased. The oncometer tracing showed that the intestinal blood vessels are constricted. Further exposure of the intestine to the action of light causes a lowering of blood-pressure and death of the animal in three to five hours.

Fig. 4. Cat. Chloralose. Balloon in intestine which can be seen by a glass window in abdomen. Light is without action on intestinal movement. Injection of 2 c.c. hæmatoporphyrin given intravenously = slight decrease of peristalsis. The figure shows the subsequent action of light. Time = 30 sec.

If the uterus of an anæsthetised cat is arranged so that its contractions can be registered, then neither the exposure of the organ to light (150 c.p. 40 cm.) nor the intravenous injection in the dark of 0·02 grm./kg. of hæmatoporphyrin has any effect on the contractions: but the exposure of the hæmatoporphyrin-sensitised uterus to light causes distinct contraction of the uterus.

Similarly the spleen volume of the rabbit is not changed by an exposure to light of 150 c.p. 30 cm.: also the intravenous injection of 0·03 grm./kg. of hæmatoporphyrin in the dark causes only a slight contraction of this organ following the lowering of blood-pressure, but the subsequent exposure of the spleen to the action of light, 150 c.p. 30 cm., causes marked constriction.

If a rat is injected subcutaneously with 0·1 grm./kg. of hæmatoporphyrin and 45 minutes later the uterus and intestines are placed in Ringer's solution so as to record contractions, they respond to visible light, though not in a marked degree.

The action of light on skeletal muscle of the frog.

The isolated gastrocnemius-sciatic nerve-muscle preparation of the frog is not sensitive to the action of visible light. A solution of hæmatoporphyrin of 1/50,000 causes a slight decrease of nervous irritability. The exposure of the hæmatoporphyrin-sensitised preparation to the action of light (150 c.p. at 25 cm.) causes slight contraction of the muscle and a slow decrease of the contractibility to stimulation.

After 30 minutes the muscle is dead. Visible light seems to have no action on the irritability of hæmatoporphyrin-sensitised motor nerve.

The action of light on cardiac muscle. Amsler and Pick(11) showed that moderate exposure to visible light of the hæmatoporphyrin-perfused heart of the frog causes a heart block. In my experiments isolated rabbit's hearts perfused with 1/3000 hæmatoporphyrin solution in Ringer's fluid were used.

Light alone (150 c.p. at 10 cm.) has no effect on the isolated heart of the rabbit. Hæmatoporphyrin solution causes an increase of the heart's activity. The exposure to light of hæmatoporphyrin-perfused heart causes a constriction of the coronaries, slowing of heart rate and a decrease of heart contraction. Ten to fifteen minutes later the heart is dead. Less intensive light (150 c.p. at 22 cm.) causes irregularity of heart beat.

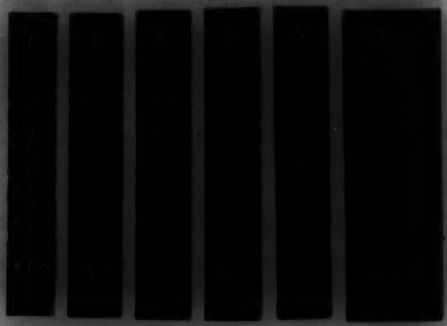

Fig. 5. Isolated rabbit's heart perfused with Ringer. 1. Normal. 2. After hæmatoporphyrin 1 in 3000. 3. Two minutes after application of light arranged 11 cm. from heart. 4. Five minutes after light. 5. Seven minutes after light. 6. Ten minutes after light. The lower figures are the rate of flow through the coronaries per minute. Time = 30 sec.

Further experiments were made on the cat's heart *in situ*. Cats were anæsthetised with urethane and the heart placed in a glass oncometer

so that it could be exposed to direct action of light. Such light shows no change in the cardiac beat. The intravenous injection of 0·02 grm./kg. of hæmatoporphyrin solution causes a transient weakening of heart muscle, lowering of the blood-pressure and a slight increase of the heart contractions. But when the heart is exposed to the action of light (150 c.p. at 30 cm.) it shows a gradual but constant decrease of contraction, an increase of the volume through weakening of cardiac muscle: this effect is associated with a steady fall of blood-pressure. The cat dies 2½ hours later.

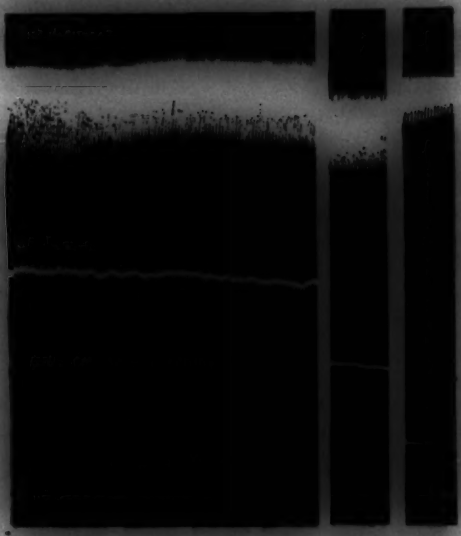

Fig. 6. Cat. A.C.E. urethane. The heart was enclosed in a glass cardiometer. Shows the action of light after hæmatoporphyrin. The separate tracings represent intervals of ½ hour and 2 hours.

The action on the blood-pressure and on the respiration.

The intravenous injection of 0·03 grm./kg. of hæmatoporphyrin causes a transient lowering of the blood-pressure. A shaved albino rabbit injected intravenously with 0·03 grm./kg. of hæmatoporphyrin and exposed to the action of light (200 c.p. 15 cm.) during a short time shows

all the symptoms of skin inflammation associated with a constant lowering of blood-pressure, diminution of the respiratory movements, decrease of blood cells and of hæmoglobin concentration. Three and a half hours later the animal dies.

Discussion.

Strong concentrations of hæmatoporphyrin cause a depression of the cellular function. The peristaltic movements are depressed, plain muscle relaxed, and the heart muscle weakened. The weakening of the heart is the cause of lowering of the blood-pressure which follows the intravenous injection of this drug. Dilute solutions of hæmatoporphyrin have no effect on the biological functions of tissues, but they sensitise them to the action of visible light. Visible light exerts on tissues an ultra-violet-like action. A prolonged exposure of hæmatoporphyrin-sensitised organs to the action of light kills them: the erythrocytes are hæmolysed, spermatozoa, protozoa, ciliated epithelium of the frog are killed, plain muscles are contracted and killed, skeletal muscles or cardiac muscles are relaxed and killed.

The light which is absorbed by hæmatoporphyrin solution is the most active. Hæmatoporphyrin-sensitised animals are killed by the action of visible light with the symptoms of skin inflammation or anæmia Kichiya(6), lowering of the blood-pressure and depression of the respiratory movements. It is difficult to judge which of these effects predominates and which is the cause of death.

Conclusions.

1. Hæmatoporphyrin sensitises isolated organs of animals to visible light. Yellow light seems to be most active.

2. Visible light causes *in vitro* and *in situ* a contraction of plain muscles that have been sensitised by hæmatoporphyrin. This contraction is dependent on the intensity of the light.

3. Visible light kills hæmatoporphyrin-sensitised hearts of warm-blooded animals.

4. Visible light kills hæmatoporphyrin-sensitised animals with symptoms of skin inflammation, anæmia, lowering of blood-pressure and depression of the respiration.

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OBSERVATIONS ON THE ETHYL IODIDE METHOD FOR THE DETERMINATION OF HEART OUTPUT.

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(*From the London School of Medicine for Women.*)

The ethyl iodide method of Henderson and Haggard(1) has aroused considerable interest and we think it may be helpful to publish some further observations on the subject. Since the appearance of our paper(2), various criticisms of the method have been brought forward, and we have realised from these and from difficulties met with by ourselves in further use of the method that considerable care is necessary in its application, if satisfactory results are to be obtained. Our own difficulties and the criticisms published are chiefly in connection with the following points:

(*a*) Estimation of the ethyl iodide.

(*b*) Collection of samples of true alveolar air.

(*c*) The return of ethyl iodide to the lung in the venous blood.

(*d*) The coefficient of absorption of ethyl iodide.

(*a*) *The ethyl iodide estimations.* Reference to Table I in our previous paper shows that the majority of circulation rates obtained do not deviate from the average by more than plus or minus 10 p.c. We were not, however, satisfied that these deviations, under the standard conditions adopted, were really due to alterations in the circulation rate, still less the occasional much greater deviations, for which no satisfactory explanation was found. This led us to enquire more closely into the method of determining the concentration of ethyl iodide in the samples of air obtained.

We first analysed duplicate samples from the spirometer, in the usual way, and found that they did not always give concordant results. This might be due to the fact that the reaction between the ethyl iodide and the pentoxide did not occur as stated by Henderson and Haggard, or it might be due to technical errors in the actual carrying out of the analyses.

[1] Working with a grant from the Medical Research Council.

Two methods of checking this first point were tried:

(1) The accurate estimation of weighed and fairly large quantities of ethyl iodide. This was done by our colleague Mr J. A. Gardner, who, on such samples, obtained a yield of iodine of about 99 p.c. of the theoretical value[1]. He therefore concluded that the reaction did occur as laid down.

(2) Estimation of very small amounts (*viz.* 1–2 mg.) such as would be present in the samples obtained during a circulation experiment. For this purpose we adopted the micropipette method described by Prof. Henderson(3) in a recent paper. Results obtained in this way were so unsatisfactory, owing presumably to the difficulties of obtaining accurate volume measurements, that we abandoned it.

In view of the findings of Mr Gardner as to the reaction, and in consideration of the fact that the method depends not upon absolute but upon comparative values, it appeared that an adequate control would be obtained if it could be shown that consecutive analyses of the same mixture produced results which agreed with each other to within 2 p.c.

For these estimations the mixture was made in a 4 litre aspirating bottle, over water, and two samples of 250 c.c. drawn off simultaneously through a Y connection.

Our first results by this method were not completely satisfactory, the figures obtained leading us to doubt whether rates of air flow through the pentoxide tube were such as to allow of the completion of the reaction.

We finally adopted the following method, which gives extremely satisfactory results for the quantities of ethyl iodide usually employed.

The air is drawn through the tube at the rate of about 200–300 c.c. a minute for the first 3 or 4 minutes to allow of complete decomposition of the ethyl iodide, and then at a litre a minute for 6 or 7 minutes, to ensure complete washing out of all the iodine formed. With stronger samples rather longer times may be necessary, and in any case a subsequent 5 minutes control blank should always be at its initial value before the analysis is considered complete. We have found it necessary to add a second absorption tube of potassium iodide, as with strong samples and a rapid flow of air some iodine is apt to be carried over from the first. Table I shows some of the figures obtained in this way.

The next control was the analysis of the two samples, each on a different pentoxide tube, and the figures so obtained are shown in Table II.

[1] Communicated result.

TABLE I. Analyses of ethyl iodide mixtures on the same pentoxide tube.

Date	Mixture and no. of sample	Size of sampling tube c.c.	Thiosulphate c.c.	Maximum p.c. difference
30. xi. 26	I. *a.*	250	5·62 } mean = 5·65	1·4
	b.	250	5·68 }	
	c.	100	2·28 in 250 = 5·70	
	II. *a.*	250	6·90 } mean = 6·82	2·2
	b.	250	6·74 }	
	c.	100	2·70 in 250 = 6·75	
	III. *a.*	250	4·20	
	b.	250	4·24	1·0
	c.	250	4·22	
2. xii. 26	IV. *a.*	250	7·70	0·3
	b.	250	7·68	

TABLE II. Analyses of ethyl iodide samples on two different pentoxide tubes.

Date	Mixture and no. of sample	Pentoxide tube c.c.	Thiosulphate c.c.	Maximum p.c. difference
20. ii. 27	I. *a.*	*A*	4·40	1·3
	b.	*B*	4·46	
	II. *a.*	*A*	3·11	0
	b.	*B*	3·11	
	III. *a.*	*A*	4·64	0
	b.	*B*	4·64	
23. iii. 27	IV. *a.*	*A*	2·54 } 2·54	1·5
	b.	*A*	2·54 }	
	c.	*B*	2·54 } 2·56	
	d.	*B*	2·58 }	

A point of some interest is the effect of alterations of rate of flow on the value of the blank. The iodine which is constantly being liberated in the tube in small quantities apparently accumulates at the slow rate of flow, and its elimination does not become steady until the flow is more than 500 c.c. a minute. During an actual analysis, however, the amount accumulated during the slow period is carried over during the rapid flow, and therefore the average blank can be used. Figures are given in Table III.

Variations in temperature also affect the blank as is shown in Table IV. We are now using an electric air bath[1] holding two pentoxide tubes. The temperature in this bath is not quite so uniform as in the oil bath, varying about 25° between top and bottom, but as the reaction occurs at any temperature between 160° to 200° this is immaterial. The temperature in the middle of the bath should be 180° to 190° C.

[1] Supplied by the British Drug Houses, Ltd.

TABLE III. Effect on the blank of varying rates of air flow through analysis train.

Time a.m.	Blank in c.c. Thio. for 5 min.	Rate of air flow through pentoxide tube
11·00	0·05	100 c.c.
11·05	0·12	500 c.c. per min.
11·10	0·11	Very rapid (over 1 litre per min.)
11·30	0·06	300 c.c. per min.
11·35	0·06	150 c.c. per min.
11·40	0·16	Very rapid
11·45	0·10	"
11·50	0·10	"

TABLE IV. Effect of temperature on the blank.

Mean temperature of bath	Blank in c.c. thio. for 5 min.
185°	0·04 (4 observations)
197°	0·05 "
203°	0·08 "
213°	0·18 "

A recent paper by Starr and Gamble(4) gives an excellent method of estimating ethyl iodide by means of silver nitrate. In view of our experience, as shown by the above figures, we have not thought it necessary to give up the pentoxide method, since, with strict attention to detail, results as good as those given by the silver nitrate method can be obtained. The pentoxide method has the further advantage that the whole experiment, including the analyses of 2 Alveolar CO_2 samples and of a sample of expired air for determination of the oxygen consumption, can be completed by two workers in an hour and a half.

(*b*) *The alveolar air.* As mentioned in our preceding paper, we found much difficulty in obtaining in women true alveolar samples with the original valves of Henderson and Haggard, which increased the dead space of the subject by something over 100 c.c. We have modified these valves, so that this increase is now less than 30 c.c. With ordinary quiet breathing, there is no pronounced resistance (actual value 1–1·5 c.c. of water), but we doubt if the valves would be suitable for the greater ventilation of exercise. Table V shows the improved results obtained on subjects with low tidal air, when the smaller valves are used. Even better results are obtained by increasing the time of the experiment from the previous period of 10 minutes, to 12 or 15 minutes, so as to ensure complete washing out of the 250 c.c. sampling tube. (See Table V.)

Consideration of these figures shows that when the above precautions are taken, the Müller valve sample is usually higher than the Haldane-Priestley sample. This is owing to the fact that the latter sample is taken

at the end of the inspiratory phase, which gives a lower figure than a sample collected at the end of expiration. Usually the Müller valve sample corresponds closely with the mean of the two. (Table VII.)

TABLE V. Alveolar CO_2 p.c. values with large and small valves.

Subject	Müller sample		Haldane-Priestley	p.c. error
J. C.	4·73	large valves	5·64	−16
Av. Tidal air : 310 c.c.	4·85		5·57	−13
	5·53	small valves	5·73	−3·5
	5·39		5·75	−6·00
	5·18		5·10 (?)	+1·5
W. C. C.	4·31	large valves		
Av. Tidal air : 400 c.c.	4·18		Av. 5·28	−18 approx.
	4·47			
	5·00	small valves	5·15	−3
	5·36		5·38	0
	5·18		5·33	−3

TABLE VI. Effects on alveolar CO_2 p.c. values of incomplete washing through of sampling tube.

Subject	Duration of experiment min.	Müller valve p.c.	Haldane-Priestley p.c.
W. C. C.	15	5·46	5·26
420 c.c. Tidal air	15	5·44	5·51
G. K.	10	5·18	5·92
480 c.c. Tidal air	12	5·51	5·91
	15	6·00	5·91
O. R.	10	4·47	4·60
800 c.c. Tidal air	15	4·81	4·70

TABLE VII. Alveolar CO_2 percentage.

	Haldane-Priestley			
Subject	End of inspiration	End of expiration	Mean	Müller
O. R.	5·09	5·64	5·37	5·34
	4·72	5·08	4·90	4·93
	4·37	4·94	4·67	4·50

We have also taken alveolar samples with the method suggested by Moore and Kinsman(5), and in the case of trained subjects obtained satisfactory results, though it is extremely difficult to prevent over breathing, and the consequent excessive washing out of the CO_2. In view of the satisfactory results obtained by our routine method, we have not considered it necessary to adopt any other.

(*c*) and (*d*). *The return of ethyl iodide to the lung in the venous blood, and the coefficient of absorption of ethyl iodide.* We have ourselves made

no attempt to determine the coefficient of absorption of ethyl iodide in blood, nor have we estimated the amount of ethyl iodide returned in the venous blood. We have so far accepted for our work the findings of Henderson and Haggard, as given in their original paper. These were checked by them *in vivo* by experiments on dogs, whereas the widely different figures given by Starr and Gamble were only obtained *in vitro*. Their paper, however, does put forward a serious criticism, and further work on this important point is clearly necessary before the method can be accepted as satisfactory for estimation of the circulation rate under widely differing conditions. Nevertheless the results obtained by us under more or less standard conditions show such close agreement that it appears that any correction which, in the light of further study of the method may be shown to be necessary, will be such as to affect absolute values, but not comparative values. As an instance of this we quote the following figures. (Table VIII.)

TABLE VIII.

Subject and conditions of experiment	Date and time		Circulation litres per min.	Stroke volume c.c.	Stroke index c.c. per kg.	Oxygen consumption c.c. per min.
O. R. Age 37; weight 49 kg. Lying flat. Average pulse 61	15. iii. 27.	10.10 a.m.	6·65	107	2·18	164
		12.20 p.m.	6·55	111	2·26	—
	16. iii. 27.	10.15 a.m.	6·83	110	2·25	176
		12.10 p.m.	6·32	106	2·16	173
	17. iii. 27.	10.30 a.m.	6·50	110	2·25	172
		12.20 p.m.	6·47	108	2·20	164
Av. of 6 experiments			6·55	109	2·22	170
Average deviation in circulation rate = 2 p.c. Maximum deviation = 4 p.c.						
E. D. Age 23; weight 62 kg. Lying flat. Average pulse 63	21. ii. 27.	10.15 a.m.	8·30	130	2·10	214
		10.50	8·00	129	2·08	216
	22. ii. 27.	10.25	8·60	140	2·26	211
	23. ii. 27.	10.20	8·50	130	2·10	218
		10.40	7·85	129	2·08	206
Average of 5 experiments			8·25	132	2·12	213
Average deviation from mean circulation rate = 3 p.c. Maximum deviation = 5 p.c.						
Same subject sitting upright Average pulse 69	24. ii. 27.	10.20 a.m.	6·05	82	1·32	215
		10.40	5·32	78	1·26	214
	28. ii. 27.	10.40	5·45	77	1·24	194
		11.15	5·90	85	1·37	204
	1. iii. 27.	10.40	5·30	77	1·24	202
		11.10	5·25	78	1·25	218
Average of 6 experiments			5·54	79·5	1·28	208
Average deviation circulation rate = 5 p.c. Maximum deviation = 9 p.c.						
Same subject standing. Average pulse 91	9. iii. 27.	12.40 p.m.	4·30	53	0·85	202
	10. iii. 27.	10.45 a.m.	4·37	45·5	0·73	202
		12.30 p.m.	4·12	43	0·69	212
Average of 3 experiments			4·26	47	0·76	205

These figures show a constant variation in the circulation rate as between the lying, sitting, and standing positions. In the case of *E. D.* this difference is considerable, perhaps owing to the fact that this subject has a very bad peripheral circulation. The same type of change has, however, already been recorded by Collett and Liljestrand(6), using the Krogh-Lindhard method. It is also referred to by Henderson and Haggard in their original paper. The average figures given for the same subject in our preceding paper were: Circ. rate 7·18 litres, Stroke vol. 108 c.c., Stroke index 1·80; and for *O. R.* Circ. rate 6·30 litres, Stroke vol. 88 c.c., Stroke index 1·80. These estimations were made in a semi-reclining position in a deck chair. A further interesting point shown in this table is the close agreement of the figures for the stroke index of the two subjects *E. D.* and *O. R.*

An interesting paper on the measurement of circulation rate by the ethyl iodide method, by Davies and Gilchrist(7), has just been published in the *Quarterly Journal of Medicine*. These authors fail to find any relationship between the circulation rate and any other physical measurements in different individuals, and therefore they consider it impossible to predict the circulation rate of any given individual. No reference however is made to the stroke index (c.c. per beat per kg. body weight) which we have found to be the most constant factor. It is also possible that the experimental conditions adopted by them are not sufficiently constant, considering the great possible variation in the circulation rate which may occur with change of posture. Referring to our previous series of experiments published last year, we find the average stroke index for 11 women under constant conditions to be 1·74 (excluding *J. C.* whose alveolar samples were never satisfactory on the large valves). The average deviation from the mean in this series is 6 p.c. and the max. deviation 15 p.c. These figures were obtained with the subjects in the semi-recumbent position in a deck chair instead of lying quite flat as in our recent work.

It will be seen that in the majority of subjects examined, the stroke volume does not vary very greatly. The cause for such variations as do occur has not been determined, but it appears that the higher stroke index occurs in the more athletic types and also in subjects who are persistently under weight, while the low figures occur in those who lead a more sedentary existence or who are over weight. As an example, we quote *M. E.*, who has a stroke index of 2·00, the highest of this series. Height 162 cm., weight 63 kilos.—athletic and a member of the rowing crew. On the other hand *J. B.*, height 162 cm., weight 65 kilos., the lowest

of the series, has a stroke index of 1·50. This subject is abnormal in various respects, and under conditions of experiment always had a pulse rate of over 100.

The number of subjects so far examined is, however, too small for us to be able to draw any definite conclusions from them.

With regard to certain technical modifications made by these authors, we note with interest the substitution of red lead coated flexible metal tubing, in place of rubber tubing, in the breathing circuits. We have not ourselves used metal tubing in our experiments, but have substituted ordinary straight rubber tubing for the non-collapsible corrugated rubber tubing. This offers a much smaller absorbing surface, although it cannot be considered entirely free from error. The rubber is further reduced to a minimum, by introducing wide bored glass tubing where possible. The relative values are not greatly affected in any case, as the length of rubber tubing used is always the same.

Summary.

In the further application of the ethyl iodide method for the determination of the circulation rate in man certain difficulties were encountered. It was found that these could be obviated by slight modifications in technique. This paper gives an account of the modifications applied to the estimation of the ethyl iodide samples, and to the collection of the alveolar air samples. Figures are given showing the greater consistency of the values obtained for the circulation rate, when these modifications are used.

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THE ACTION OF HIRUDIN UPON THROMBIN.

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It has been shown that hirudin retards the coagulation of fibrinogen by acting upon thrombin (Gratia(1), Barratt(3)). As a result of this action a portion of the thrombin originally present disappears. The amount disappearing is not, however, proportional to the hirudin present: on the contrary, a considerable increase of the quantity of hirudin added causes only a relatively smaller increase of the amount of thrombin disappearing (cp. Table I).

A similar relation obtains in adsorption phenomena and is also observed in connection with hydrolytic dissociation. It was therefore decided to investigate whether the disappearance of thrombin upon the addition of hirudin could be interpreted as due to adsorption or as indicative of dissociation.

The results of experiment will be considered in respect of: (1) the adsorption of thrombin by hirudin; (2) the formation of a dissociated compound between thrombin and hirudin of the strong acid—weak base or the weak acid—strong base type; (3) the formation of a dissociated compound of the type weak acid—weak base.

The equations of equilibrium for these three cases are:

$$c = p\left(\frac{m-c}{d}\right)^q \qquad (1),$$

$$c(df - m + c) = H(m - c) \qquad (2),$$

$$c(df - m + c) = H(m - c)^2 \qquad (3),$$

where d and m represent the total amounts of hirudin and thrombin respectively added, c is the quantity of thrombin remaining free and p, q, f and H are constants[1].

[1] Equations (2) and (3) may be given in the form

$$\frac{[\text{acid}] \times [\text{base}]}{[\text{salt}]} = \text{const.}$$

and

$$\frac{[\text{acid}] \times [\text{base}]}{[\text{salt}]^2} = \text{const.}$$

respectively, the quantities in brackets representing molecular concentrations. Since the

For experimental work the above equations are more conveniently written

$$c = pC^a \qquad (1\,a),$$

$$c(f - C) = HC \qquad (2\,a),$$

$$c(f - C) = Hd C^a \qquad (3\,a),$$

where C represents $\frac{m-c}{d}$. When in this form it is easy to make a preliminary choice of equation by referring to the results of experiment, for it is seen that in equations (1 *a*) and (2 *a*) a single value of C corresponds to a given concentration of free thrombin (c) and is independent of the respective amounts of hirudin and thrombin employed to obtain this concentration: that is to say, the same value of C is obtained no matter whether relatively large or relatively small amounts of hirudin and thrombin have been added in order to give the concentration of c observed. On the other hand, when the dissociation is of the type given by equation (3 *a*), the value of C corresponding to a given value of c is not fixed but varies in each experiment, being dependent upon the concentrations of hirudin and thrombin added.

Before proceeding to a consideration of experimental results a brief reference may be made to the form of the curves corresponding to equations (1 *a*), (2 *a*) and (3 *a*) (cp. Barratt(2)). These are given in Figs. 1, 2 and 3 respectively, in which ordinates represent c and abscissæ C. The axis of abscissæ, it will be seen, is a tangent to curves 1 and 3 at the point of origin of co-ordinates. Curve 1 has no asymptotes, but curves 2 and 3, which are hyperbolic, have one asymptote in common, given by the equation $C = f$, indicated by the thick vertical line on the right of Figs. 2 and 3. The length of curve investigated affords an

molecular concentrations of hirudin and thrombin are unknown, the actual amounts of solid material contained in the solutions of these substances employed are given instead, a dilution factor f being introduced, so chosen that df indicates the molecular concentration of d relative to the unknown concentration of m taken as a standard. If free base is represented by c, and m and d represent the *total* amounts of base and acid respectively added, it will readily be seen that the above equations assume the form given in (2) and (3).

If equation (3) is applicable to experiments made in the presence of plasma, a buffered fluid, it would follow that the dissociation constants of the weak acid and weak base must be represented by the H^+ and OH^- concentration of plasma respectively, the free weak acid and weak base being unionised but the combination of the two (thrombin-hirudin) ionised.

indication as to whether the range of experiment is likely to be sufficiently extensive to enable the type of curve to be identified. All experiments

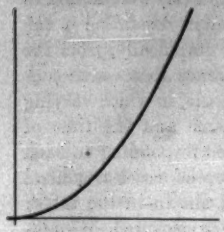

Fig. 1.

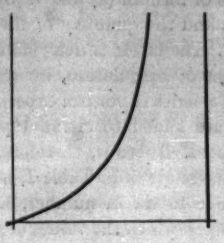

Fig. 2.

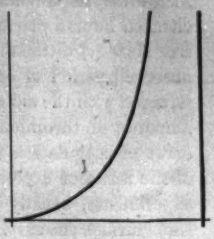

Fig. 3.

corresponding to equations (1 *a*) and (2 *a*), in which there are only two variables *c* and C, lie upon the curves given in Figs. 1 and 2 respectively. On the other hand, experiments corresponding to equation (3 *a*), which has three variables *c*, C and *d*, lie upon a curved surface: curves of the type shown in Fig. 3 represent fixed values of *d*.

In Table I the results of 20 experiments carried out at room temperature (16° C. to 18° C.) are given. These fall into five groups, the

TABLE I.

Experiment	*d* Total hirudin added	*m* Total thrombin added	*c* Free thrombin
1	4830×10^{-6}	$9{\cdot}47 \times 10^{-6}$	$0{\cdot}063 \times 10^{-6}$
2	458	9·47	0·44
3	,,	2·37	0·031
4	,,	0·592	0·0034
5	162	9·47	1·67
6	,,	2·37	0·070
7	,,	0·592	0·010
8	47·2	9·47	4·0
9	,,	2·37	0·50
10	,,	0·592	0·020
11	37·9	9·47	5·4
12	,,	2·37	0·29
13	,,	0·592	0·034
14	9·47	9·47	7·8
15	,,	2·37	1·22
16	,,	0·592	0·24
17	2·37	9·47	9·2
18	,,	2·37	1·75
19	,,	0·592	0·05
20	,,	0·148	0·040

first consisting of a single experiment, the second, third and fourth groups each of three experiments, and the last of ten. In these experiments varying amounts of hirudin (d) and thrombin (m) were added to citrated human plasma and the quantity of thrombin remaining in the free state (c) estimated. The latter is determined graphically, from the observed period of onset of coagulation, by reference to a curve constructed with the aid of a series of control experiments, in which varying amounts of thrombin are added to citrated plasma and the time of onset of coagulation noted. There is, therefore, corresponding to each of the series of experiments given in Table I, a second series of control experiments, usually four to six in number, not shown in the table: these furnish the curve upon which the values of c are measured. Hirudin and thrombin, it may be observed, are represented by the amount of solid material contained in the quantities of their respective solutions present in different experiments: in the tables the amounts given are those contained in 1 c.c. of the mixture of plasma, hirudin and thrombin employed.

TABLE II.

Experiment	d	m	c	C
4	458×10^{-6}	$0{\cdot}592 \times 10^{-6}$	$0{\cdot}0034 \times 10^{-6}$	0·00129
7	162	0·592	0·010	0·00359
10	47·2	0·592	0·020	0·0121
3	458	2·37	0·031	0·0051
13	37·9	0·592	0·034	0·0147
20	2·37	0·148	0·040	0·0455
1	4830	9·47	0·063	0·0020
6	162	2·37	0·070	0·0142
16	9·47	0·592	0·24	0·0372
12	37·9	2·37	0·29	0·0550
2	458	9·47	0·44	0·0197
19	2·37	0·592	0·50	0·0388
9	47·2	2·37	0·50	0·0396
15	9·47	2·37	1·22	0·1213
5	162	9·47	1·67	0·0482
18	2·37	2·37	1·75	0·2620
8	47·2	9·47	4·0	0·1160
11	37·9	9·47	5·4	0·1074
14	9·47	9·47	7·8	0·1765
17	2·37	9·47	9·2	0·1140

In Table II the values of C corresponding to the experiments given in Table I are shown. For the sake of convenience these experiments are re-arranged in order of the value of c. When corresponding values of c and C are compared it is found that the latter are dependent upon the amounts of hirudin (d) and thrombin (m) added: for approximately

equal values of c, C increases as d and m decrease. This is strikingly exhibited by Exps. 3, 13 and 20 and also by Exps. 1 and 6, Exps. 2 and 19 and Exps. 5 and 18. In other words, when the amounts of hirudin and thrombin employed to furnish a given value of c are large, C will be small, while if small amounts are used C will be large.

Since the value of C corresponding to a given value of c is not fixed, but varies with the amounts of hirudin and thrombin employed, it follows that equations (1 a) and (2 a) are not applicable to the experiments given in Table I: that is to say, hirudin does not adsorb thrombin, nor does it enter into chemical combination with thrombin to form a dissociated compound of the type corresponding to equations (2) and (2 a). On the other hand, these experiments are in agreement with equation (3 a), so far as the variability of C is concerned. It remains now to determine whether constants corresponding to equation (3 a) can be obtained for the experiments made.

TABLE III.

Experiment	c obs.	c calc.
1	$0{\cdot}063 \times 10^{-4}$	$0{\cdot}063 \times 10^{-4}$
2	0·44	0·66
3	0·031	0·041
4	0·0034	0·0026
5	1·67	1·80
6	0·070	0·12
7	0·010	0·007
8	4·0	4·8
9	0·50	0·39
10	0·020	0·025
11	5·4	5·4
12	0·29	0·48
13	0·034	0·031
14	7·8	8·2
15	1·22	1·35
16	0·24	0·12
17	9·2	9·1
18	1·75	2·04
19	0·50	0·34
20	0·040	0·030

The calculation of the constants by ordinary algebraical methods is found to be so seriously affected by even slight errors in the estimation of c as to render this mode of determination impracticable. The only method available is to assume a series of arbitrary values for H and then to ascertain if any of these will furnish values of f and c in fair

agreement with experiment. After many trials the value of H was taken at 0·5 and that of f at 0·1484, whence the values of c given in the third column of Table III were calculated.

In view of the difficulty, among other sources of error, of determining the precise moment at which the onset of coagulation occurs, the extent of agreement exhibited between observed and calculated values of c in Table III, extending over a range of concentration of thrombin in the proportion of 64 to 1 and of hirudin in that of 2000 to 1, must be regarded as satisfactory, and indicates that the interaction of hirudin and thrombin is the same as that of weak acid and weak base. This conclusion is further supported by the circumstance that the same value of H is applicable to all the different series of experiments given in Table I. The value of H, it may be observed, is dependent upon the relative, not the absolute, concentrations of thrombin used in the different series, so that deterioration of the stock solution of thrombin employed occurring in the intervals of time elapsing between these series of experiments, does not affect the determinations of H furnished by the individual groups of experiments in Table I.

On the assumption that the values of the constants given above have been correctly selected the range of experimental error is exhibited by Table III. In this table the probable percentage variation of a single estimation of c from the calculated value is found to be approximately $\pm$ 20 p.c. The range of Exps. 8, 9 and 10, it may be observed, is represented by the length of curve in Fig. 3.

Since the experiments made conform to the type of equation (3) the further conclusion follows that both hirudin and thrombin are in a state of true solution in the dilutions employed: there is nothing to suggest the presence of a disperse phase.

Summary.

The experiments made indicate that:

(1) thrombin is not adsorbed by hirudin;

(2) thrombin and hirudin do not form a chemical combination of the weak acid—strong base or strong acid—weak base type;

(3) the interaction of thrombin and hirudin is the same as that occurring when a weak acid and a weak base are mixed together, part of each entering into chemical combination, the remainder being free;

(4) in the concentrations employed hirudin and thrombin are in true solution.

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THE INFLUENCE OF POSTURE ON THE VOLUME OF THE RESERVE AIR.

By WILLIAM H. WILSON.

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The purpose of this communication is to draw attention to certain facts regarding the effect of posture on the volume of the lungs, as indicated by measurements of the reserve air, of which I can find no record in the literature of the subject, and to their physiological significance.

Introduction. "Reserve Air" is the term originally applied by Hutchinson(1) in 1846 to that volume of air which can be expelled at will from the lungs after the end of a normal expiration. It is stated by this author to have an average volume in adult men of 100 cubic inches (1560 c.c.), the corresponding volumes of the tidal and complemental being respectively 20 and 110 cubic inches (312 and 1710 c.c.).

The figures given in most English textbooks (*e.g.* Starling(2)) are 1500 for the reserve air, 500 for the tidal and 1500 for the complemental air. Boruttau(3) gives reserve air, 1600 c.c.; tidal air, 500; complemental air, 1600. It is of course now evident that the tidal air varies with the rate of production of CO_2 by the body at the time.

Hutchinson(4) records that the vital capacity is less in the recumbent than in the erect posture: he gives the following figures in his own case: Standing 260 cubic inches, sitting 255, recumbent supine 230, and prone 220, a difference of about 600 c.c. Whether the diminution affected the reserve or the complemental air is of course not shown[1].

Observations which seem to show that considerable variations in the volume of the reserve air are found in different individuals, apart from differences in their respective vital capacities, have been made by Flack(5) in flying officers. Flack examined 69 pilots and found volumes for the reserve air varying from 26 to 55 p.c. of the vital capacity volume. The mean figures of all the observations were, reserve air, 1560, vital capacity 3950, the minimum being 1050, the maximum 2100 c.c. That the difference was not due to weakness of the expiratory muscles seems to be indicated by the fact that the "expiratory force" was normal in both subjects, the one giving the maximum, the other the minimum

[1] In observations of the vital capacity made on others and myself no marked difference has been noted in different postures.

figure. It is to be noted that the subjects were all examined under the same conditions. There is, in my opinion, little doubt that the variations noted by Flack are largely due to the difficulty of obtaining reliable measurements in inexperienced subjects.

Flack's observations refer to different individuals, but I can find it nowhere indicated that in normal persons there are variations in the reserve air volume corresponding to different positions in the same individual. The method usually adopted in measurements of the pulmonary capacity is perhaps responsible for this. It is in fact the same as that originally described by Hutchinson(1) in his directions for the use of the spirometer. The directions were "...let the person to be examined loose his vest, stand perfectly erect, with the head thrown well back,..." The posture is that which for convenience I have called "standing at attention" and is well shown by diagram 26 in Hutchinson's original paper. As regards the measurement of the reserve air it is usually directed that after the end of a quiet breath a maximum expiration should be made into the spirometer, the volume so expelled being observed.

Method. To demonstrate and record changes in the position of the tidal exchange relative to the two extremes of ventilation and the corresponding changes in the volume of the reserve air, I have employed a recording spirometer of the Hutchinson type. The spirometer is arranged for rebreathing, being provided with inlet and outlet tubes connected through valves to a Douglas mouth-piece. On the inlet passage is a chamber containing pumice and sulphuric acid, the purpose of which is to enlarge the total air space of the apparatus and to act as an air-filter, there being a certain danger of infection from one individual to another if some such device is not adopted. The movements of the spirometer chamber are transmitted to a lever recording on a kymograph. The volume corresponding to a given movement of the writing point having been determined, volumes may be accurately measured on the completed record.

To conduct an observation, the mouth-piece is fixed in position and the subject instructed to breath through the nose. When the record is to begin the subject is told to put on the nose-clip. He is then allowed to breathe quietly for about 20 seconds, at the end of which time he is told to fill the chest to the fullest possible extent and then expire as deeply as possible; after which he continues breathing quietly until the end of the observation, when he is instructed to inspire and expire again as deeply as possible.

A line joining the points of maximum expiration at the beginning and end of the record gives the base line from which the volume of the reserve air is determined. From this line measurements are made to the lowest points of the record of breathing in any posture.

If it be desired to compare the volumes of the reserve air in different postures successively in the same experiment, as in the example shown (Fig. 1), the subject must be instructed previously as to the procedure to be adopted.

The following figures are reproduced from records obtained in the manner described.

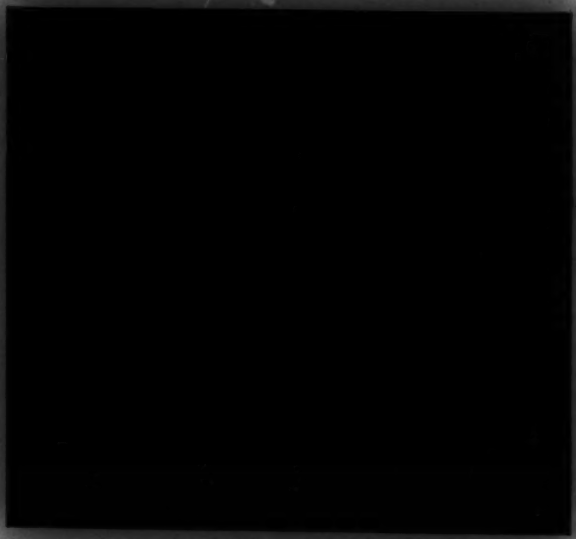


Fig. 1. Spirometer record of respiration in different postures.
Vital capacity: 3500 c.c. Subject W. H. W.

Posture	Reserve air
1	1150
2	950
3	700
4	1550
5	1550

Direction of record: right to left. Time mark: 6 secs.

Fig. 1 illustrates the effect of a series of postural changes in the course of a single observation. The numbers mark the changes of posture, which were as follows:

1. Standing in an easy attitude with one hand resting on table.
2. Sitting in chair.
3. Recumbent on back, legs extended.
4. Sitting erect in chair with shoulders well back.
5. Standing at attention, head up, hands at side.

The records reproduced in Fig. 2, *A* and *B*, are from the same subject taken about 18 months later than Fig. 1. They illustrate the effect of the recumbent posture in *B* as compared to the erect posture in *A*, the posture in both cases being maintained unchanged during the period of the observation. The vital capacity is the same in each, namely 3430 c.c.; while, however, the reserve air volume in the erect posture, *A*, is 1430 c.c., in the recumbent posture, *B*, it is reduced to 240 c.c.

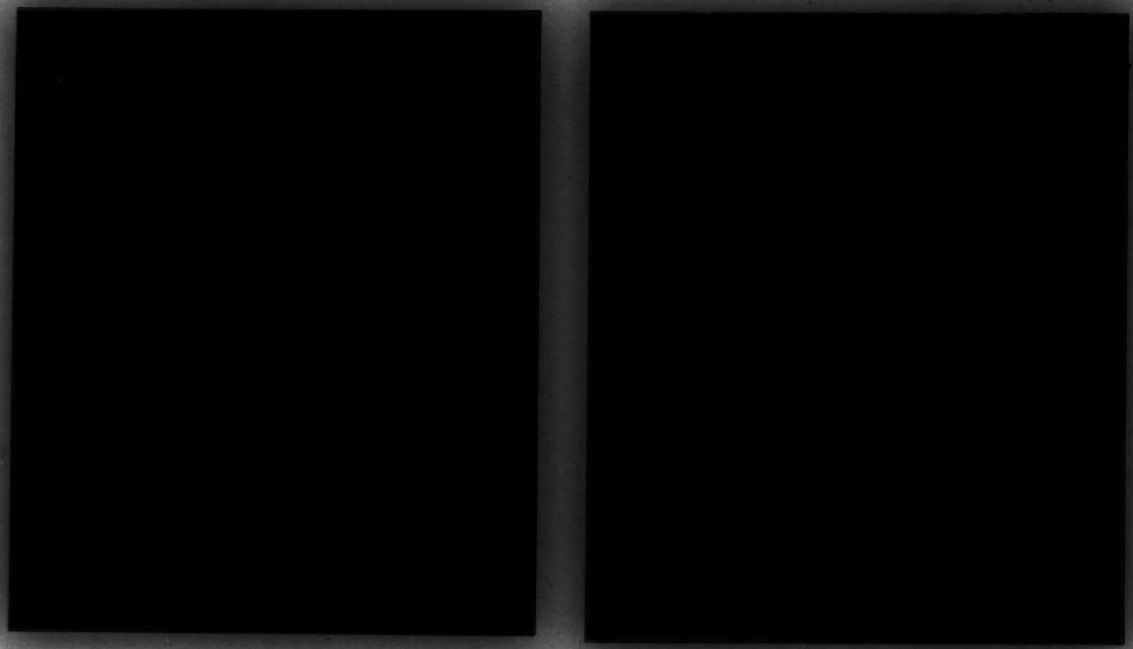
It is to be noted that while in the earlier observations, of which Fig. 1 is a record, the subject lay on three chairs with the head and shoulders supported and the legs extended in a rather strained position, in later observations, of which the records shown in Fig. 2 are examples, the subject lay on a couch with the head only supported and the knees slightly drawn up. The muscular relaxation obtained with the more comfortable arrangement was more complete; a fact which may explain the considerable difference in the reserve air in observation 2 *A* as compared to 1.

Note. The measurements are not corrected for temperature. As, however, the air temperature in the laboratory (in Cairo) was about 86° F. in each case no important difference would result.

The records shown in Figs. 1, 2 *A*, and 2 *B* are from observations on myself. As will be seen from the following table experiments on four other healthy subjects aged respectively 35, 30, 27 and 39 years, gave a similar result, although not with a minimum for the reserve air so low as that seen in Fig. 2 *A*.

	Vital capacity	Reserve air	
		Erect	Recumbent
A. H.	3900	1600	650
R. W.	4400	1700	700
A. J.	5500	2400	800
R. H.	4160	1460	590

Result. Figs. 1, 2 *A*, and 2 *B*, demonstrate sufficiently clearly that the air content of the lungs may be more than a litre less in the recumbent



A B

Fig. 2. Subject W. H. W. Vital capacity: 3430 c.c. *A*, posture erect; reserve air, 1430 c.c. *B*, posture recumbent supine; reserve air 240 c.c. Direction: right to left. Time mark: 10 secs.

than in the erect posture, and that with every change in the position of the body the base-line of normal respiration shifts to a different level.

By the expression "the base-line of respiration" is meant the level reached at the end of a normal expiration as indicated by a line joining these points on a record. That this level is unaffected except by change of posture is shown very well in Fig. 3, the record of an observation in which the subject remained sitting, leaning slightly against the back of a chair, throughout.

Fig. 3. Spirometer record. Subject sitting in chair.
Expiration, below; inspiration, above.
Vital capacity: 3400. Reserve air: 1000 c.c.
Complemental air: beginning, 1770; end, 1330.
Duration of experiment: 2 mins. 10 secs.
Time mark: 10 secs.

It will be seen that the volume of the reserve air remains constant, the line joining the points of maximum expiration being parallel with the line joining the terminations of individual expirations, although, owing to hyperpnœa due to the accumulation of CO_2, the depth of inspiration is 450 c.c. greater at the end than at the commencement.

The fact that in dyspnœa, even when the inspiratory hyperpnœa almost reaches the extent of a maximal inspiratory effort, the volume

of the reserve air is unaffected except by posture[1], can be readily demonstrated in a rebreathing observation with the recording spirometer. If any considerable acceleration in the rate of breathing accompanies the hyperpnœa there is a slight shifting of the base-line upwards in the direction of inspiration with a corresponding increase in the reserve air.

The maintenance of the base-line in a fixed position, however considerable the volume of the tidal air, is related to the essentially passive character of expiration as compared to the almost entirely active character of inspiration. The rise of this base-line in rapid breathing may perhaps be regarded as an indication that the elastic recoil of expiration requires a certain time for the expulsion of air against the frictional resistance of the air-passages, the inspiration thus commencing at a higher level than would normally be the case.

The base-line of respiration in any posture is evidently determined by the point at which the various passive forces acting on the ribs and diaphragm are for that posture in equilibrium. The act of inspiration disturbs this balance of forces; at its cessation equilibrium is restored.

In connection with the observations recorded above it is of interest to consider the postural changes in the level of the diaphragm. Postural changes with the trunk vertical, as in sitting or standing, must affect primarily the position of the ribs, and secondarily, through an increase or decrease of the lower costal circumference, the position of the diaphragm. In the recumbent posture, whatever may be the position of the ribs, the fact of primary importance is the increase in the subdiaphragmatic pressure from + 5 in the erect to + 16 (Keith(6)) in the recumbent position of the body.

I have to thank Prof. Elliot Smith for giving me the necessary facilities, and Mr Melville, of the X-Ray Department of the Anatomical Institute at University College, London, for making skiagrams of the thorax under various conditions of respiration corresponding to the spirometer records shown; and also Dr Sankey (Radiologist to the Radcliffe Infirmary, Oxford) for taking three similar skiagrams of the thorax in the recumbent supine posture. I was myself the subject.

Fig. 4 is a reproduction on reduced scale of a tracing on one sheet from five films (I, II, III, IV and V). The broad dark lines show well the

[1] Whether this is equally true of the hyperpnœa of muscular exertion is not indicated by the experiments detailed in this paper. If, however, there be a diminution, apart from postural changes accompanying the exercise, it implies that an active factor takes part in expiration under such conditions which is not present during rest or in the hyperpnœa produced by a moderate excess of CO_2 in the air breathed.

relative position of the dome of the diaphragm in the different postures at the end of a normal expiration, the level rising about half an inch in

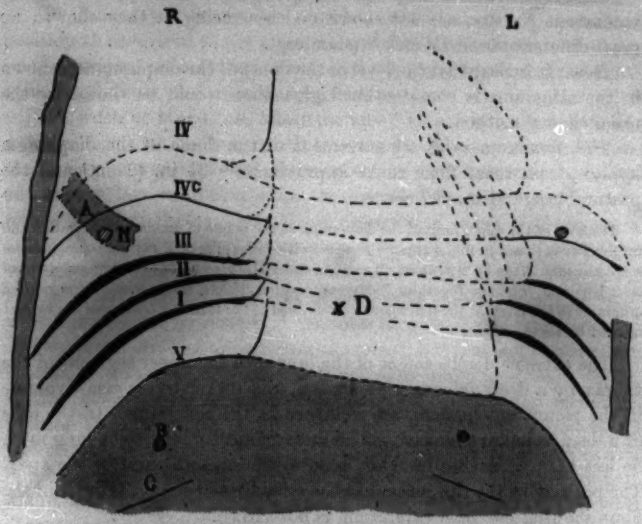


Fig. 4. Tracing from skiagrams of thorax showing level of diaphragm under different conditions.

I. End of normal expiration, subject standing erect.
II. End of normal expiration, subject standing easy.
III. End of normal expiration, subject recumbent supine.
IV. Dome of diaphragm, maximum expiration, subject standing.
IV *C*. The same corrected for level of tube (*D*).
V. Maximum inspiration (uncorrected).
A. 4th rib. *N*. Nipple. *B*. Point four inches below nipple.
C. Approximate line of costal margin. *D*. Level of tube focus.
The broken lines on the left show outline of heart.

passing from the first to the second and a further inch in the recumbent posture (III). The position of these lines needs no correction, the focus of the tube being approximately level with the highest point of the diaphragm. The upper broken line (IV) shows the actual position of the shadow of the diaphragm, the lower continuous line (IV *c*) the corrected position, in maximal expiration. The lowest line (V) gives the level of

the diaphragm in the fullest possible inspiration. (Corrected, this would be slightly higher.)

Skiagrams VI and VII, extreme expiration and inspiration in the recumbent posture, are not shown on the tracing as they showed no great difference from IV and V (standing).

Note. It is clear that the level of the dome of the diaphragm as shown in the skiagrams is considerably higher than would be shown by the usual clinical method.

The maximum range of movement of the dome of the diaphragm appears from these films to be approximately 3½ in. (9 cm.) and the postural range 1½ in. (3·7 cm.).

The level of the upper limit of the diaphragm at the points of maximum expiration and inspiration as shown in Fig. 4 appears to agree with the results of X-ray photographs from a healthy man given by Haldane, Meakins and Priestley (7) in the figure on p. 448 of their paper on "Shallow Breathing." My observations do not, however, show the marked difference in the levels in the recumbent as compared to the standing posture of the subject noticed by these authors.

The extreme displacement of the lung into the upper segment of the thorax by a deep voluntary expiration, and its relatively small volume in the recumbent posture, are well seen in the skiagrams. In the case of a deep voluntary expiration the measurement of the right lung must be less than six inches from the base to the highest point of the apex. The position of the ribs, except in the recumbent position III and in the films showing maximal expiration, is not shown.

The skiagrams demonstrate the same fact as the records of ventilation.

Remarks.

The conclusion which may be drawn from the above is that the usually accepted volume for the reserve air is only true of an unusually erect posture. This may be the normal posture of an exceptionally well set up vigorous man belonging, for example, to Dreyer and Hanson's Class A (8) but it is certainly not the posture of the ordinary individual. The change to the recumbent posture, it is seen, may result in a diminution of more than a litre in the volume of the reserve air. The residual air is stated to vary in different individuals from 1200 to 1600 c.c. assuming, as is probable, that 1400 c.c. corresponds to an average vital capacity of 3500, the result, as regards the volume of air which remains in the lungs and is ventilated by the ebb and flow of the tidal air, is that while in the erect posture this would amount to 2900 c.c., it might in the recumbent posture be as little as 1650.

It is clear that such considerable postural changes in the air content of the lungs should be taken into account in considering the change in composition produced by the inspiration of a given volume of air.

It will be seen, if the above figures be accepted, that in the recumbent position as compared to the erect there would be a diminution of 40 p.c. in the volume of the lungs, which must be accompanied by a corresponding diminution in the alveolar surface.

The work of Haldane, Meakins and Priestley(7) showed that in different parts of the lung the ratio between alveolar ventilation and the supply of venous blood varies. Certain parts are more ventilated and others less ventilated than the average, the result being, owing to the shape of the oxyhæmoglobin dissociation curve, that the mixed arterial blood is less fully saturated with oxygen than if the same amount of air had been evenly distributed over the alveoli. They found that not only was the effect exaggerated with shallow breathing, even when the rate was so great that the total lung ventilation was increased and the mean alveolar oxygen pressure raised, but that in the recumbent posture the exaggerated effect was much more easily produced.

The observations detailed above, showing that in the recumbent position the reserve air is much reduced, are evidently related to the latter effect. It was pointed out by Keith(9) that when the lungs expand in inspiration they do not expand evenly as a whole but part by part, somewhat similarly to the opening of a lady's fan. It appears that when the reserve air is diminished, as in the recumbent position, this peculiarity is accentuated, and accounts for the "orthopnœa" so often observed in severe heart or lung disease, since the blood is less fully oxygenated in the recumbent position of the body.

It would be outside the scope of this communication to consider further the clinical bearing of the facts demonstrated, beyond indicating that the large normal variations in the level of the base-line of respiration must be related to the posture in which respiration is most efficiently carried out in persons suffering from pulmonary or cardiac affections.

Conclusions.

1. The volume of the reserve air varies with the posture of the body in a man of average vital capacity from a minimum of as little as 250 c.c. in the recumbent supine, to a maximum of 1550 c.c. in the erect position.

2. The diminution in the reserve air in the recumbent position is related to the orthopnœa observed in certain cases of chest disease.

I have to thank my friend Dr Ali Hassan for much valuable help, and Dr J. S. Haldane, F.R.S., for his kindness in reading through the draft of this paper and for his advice thereon.

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REACTIONS OF ISOLATED SYSTEMIC AND CORONARY ARTERIES.

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Recent studies of the coronary circulation on the whole animal and on the isolated heart have demonstrated certain differences between various reactions of coronary and systemic blood vessels. A number of observers (1, 2) have shown that the coronary circulation increases independently of changes in the heart rate when the blood or perfusing fluid is cooled, whereas the circulation through different denervated systemic organs increases on raising, and diminishes on lowering the temperature of the blood, or perfusion fluid (3). Experiments performed on whole organs do not permit of conclusions as to whether the differences in the reaction of blood vessels to temperature depend upon the response of the whole vascular system of the organ, or more exclusively upon the arteries or capillaries, nor still less as to whether or not the reactions are occasioned by changes in the metabolism of the organ. We resolved, therefore, to study first of all the reactions to heat of isolated coronary and systemic arteries, freed from their surrounding tissues. The arteries used were taken from animals immediately after death and placed in borate (4) or carbonate Ringer's solution of *p*H 7·50. Rings of about 2 to 4 mm. in length were cut from the arteries and connected in series with silver hooks, three rings being used in one chain, in a Dewar's flask of 50 c.c. capacity. The lever magnifying the movements 34 times was loaded by weights varying from 2 to 4 grm., according to the size of the artery. About 3 c.c. of fresh defibrinated blood were added to the solution, and a gentle stream of oxygen bubbled through it. The arterial rings were placed in the solution at room temperature and then slowly warmed by means of an electric bulb. The rate of the recording drum was 2·0 mm. per minute. Arteries which could not be used immediately were kept in oxygenated Ringer at about 0° C. Most of the experiments were performed upon arteries of the ox

and dog; a few experiments were made upon the coronary arteries of man.

TEMPERATURE REACTIONS. A distinct difference exists in the appearance of the coronary and systemic arteries as they are received fresh from the animal. At room temperature the coronary arteries are wholly relaxed, while the systemic arteries are markedly rigid.

(a) *Systemic arteries.* Temperature variations in systemic arteries were first studied by MacWilliam(5) who described a contraction of the isolated artery occurring between 25° C. and 35° C., a second contraction between 45° C. and 50° C. and a final contraction occurring at between 60° C. and 65° C. After the first and second contractions the arteries relaxed to a variable extent. Great variations from this type of temperature curve were found, according to whether the artery was relaxed or contracted when the experiment was begun. Such variations may be expected when the arteries were used some time after death, and had been kept, sometimes for 24 hours or more, in oil or on ice. The most definite result of MacWilliam's experiments was what he termed "heat contraction" which took place between 60° C. and 65° C. Meyer(6) has obtained similar results with arterial rings. Cow(7) on the whole confirmed these results, and found on warming the perfusion fluid from 12° C. to 55° C., that a preliminary slight relaxation of tone of the systemic artery was followed, at about 25° C., by a slight but transient recovery of tone; this in its turn gave place to a further progressive relaxation which continued up to 50° C., beyond which point the heat contraction of MacWilliam supervened.

In our experiments upon fresh systemic arteries of the dog (renal, carotid, femoral and mesenteric) a relaxation following a transient increase in tone, as described by Cow, was the usual result. Fresh ox arteries suspended in oxygenated borate, Ringer's solution of *p*H 7·5 invariably give the type of curve (A) shown in Fig. 1. If the heating be not too rapid the fresh artery, after a transient contraction, starting at about 19°–21° C., begins to relax between 26° and 30° C., more usually in the neighbourhood of 27° C., and by the time the temperature has been raised to 37·5° C., the lumen of the artery is equal to or even greater than that obtaining at room temperature. Only in a few cases did the systemic artery at 37·5° C. fail to relax to the room temperature level, but in these cases also the maximal contraction was at about 27°–30° C. Maintenance of the temperature at 37·5° C. leads to a still further relaxation of the vessel wall until a base line is reached, which is far below the original. The degree of relaxation of the systemic artery

depends upon the extent of the tonic contraction existing when the warming is begun. Arteries which have been kept for some time in cold

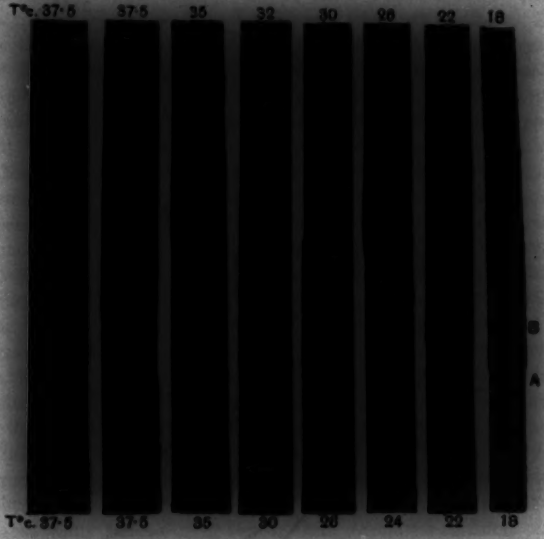

Fig. 1. Effect of warming of mesenteric (curve *A*) and coronary (curve *B*) arterial rings of the ox, about 20 minutes after death. Three rings were used together in each of the tracings; the inside diameter of the rings at room temperature was 1·9–2·1 mm.; each of the coronary rings contracted on warming by 1 mm., thus halving its diameter. Tracing to be read from right to left. Reduction $\frac{1}{3}$.

Ringer's solution, and especially in the absence of oxygen, give a considerably smaller contraction. If used about 6 to 8 hours after excision they fail to give any contraction but relax progressively as soon as the warming is begun (Fig. 2). The systemic arteries reach a maximum relaxation at about 39°–40° C. after which, and more usually at about 44° C., they again begin to contract, the contraction reaching an apex at about 48° C. In the great majority of cases this contraction did not raise the tone of the artery to that existing at room temperature, though in a few experiments the tone of the artery at 48° C. was equal to, or slightly greater than, that at room temperature.

At 48° C. or 50° C. a sudden and most profound relaxation takes

place; this continues until the temperature has reached 60° C. when the artery again contracts, never, however, reaching its original base line;

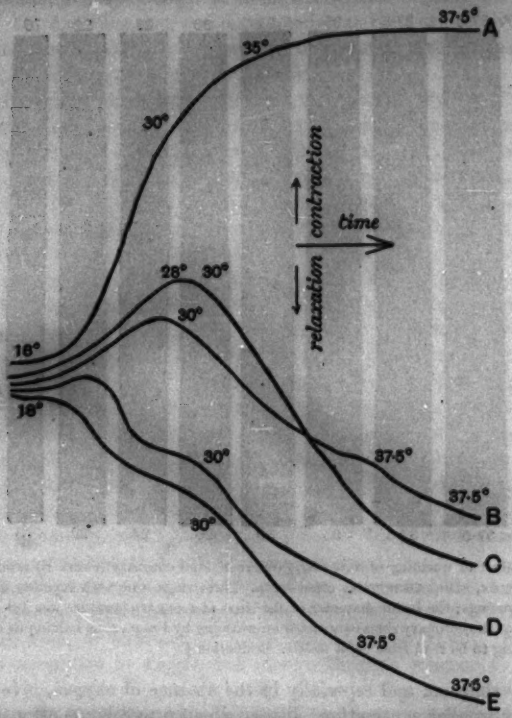

Fig. 2. Re-drawn superimposed curves; to read from left to right. Effect of warming of:
Curve A. Coronary rings, 24 hours old, kept in Ringer's fluid.
„ *B.* Mesenteric rings of the same diameter, 6 hours old, kept in oxygenated Ringer's fluid.
„ *C.* Mesenteric rings, fresh.
„ *D.* Mesenteric rings, 8 hours old, kept in Ringer's fluid.
„ *E.* Mesenteric rings, 24 hours old, kept in Ringer's fluid.
Ox arteries. Reduction ½.

further heating has no effect. The temperature reactions of the systemic rings are reversible up to 45° C. and at the latter temperature only when

it was not maintained for more than a few minutes. Approximately at this temperature the arteries cease to respond to adrenaline. The profound relaxation of arteries (at 48°–50° C.) has been described for other types of smooth muscle(8) under the name of heat paralysis. Evans states that if a guinea-pig's uterus—in which heat paralysis occurs at about 49° C.—is quickly cooled, it regains its excitability and once more responds to drugs. In the case of arteries we found no such return of excitability on cooling the rings after heat paralysis had taken place.

(*b*) *Coronary arteries.* Within the range of temperatures from 18° C. to about 38° C. the coronary arteries behave differently from the systemic. The coronary artery progressively contracts with increase of temperature from 18° C. to 37·5° C. If the heating be gradual the maximal contraction is reached at about 37·5° C. (Fig. 1 B). If, however, the temperature be raised rapidly, the contraction will continue for some considerable time after the temperature has reached 37·5° C. The time taken to raise the temperature was generally, as in the case of the systemic arteries, about 30 minutes. In arteries which are not quite fresh the uniformity of the temperature curve may be broken by a small transitory relaxation. The artery at 37·5° C. is contracted well above the base line at room temperature so that the diameter at the higher temperature may be reduced by as much as half. The artery remains contracted so long as this temperature is maintained. This contraction is reversible, and provided the artery is kept well oxygenated it can be repeated many times during two or three days after excision. In some experiments we repeated the cooling and warming of the coronary artery up to body temperature six times, in all cases obtaining the same degree of contraction. In contrast with this the systemic artery when tested a few hours after excision, fails to give even the transitory contraction described above (Fig. 2). Above 38° C. the coronary artery relaxes; from about 45° C. it behaves in the same manner as the systemic artery, *i.e.* it contracts up to 48°–50° C., then completely relaxes to the base line, and at 60° C. gives a final and vigorous contraction. The difference in the response of systemic and coronary arteries at the higher temperatures is mainly quantitative: the contraction between 45° C. and 50° C. occurs in the case of the systemic artery when it is relaxed, in the coronary when it is still in a state of partial contraction. In the first case the base line at room temperature is reached or approximated during the periods of contraction between 45° C. and 50° C. and above 60° C.; in the second it is reached or approximated during the period of relaxation between 50° C. and

60° C. This behaviour of the arteries is represented in the following table:

Changes in tone of the isolated arterial rings related to the tone of the artery at 22° C.

Temperature °C.	Systemic artery			Coronary artery		
	Below	Base line	Above	Below	Base line	Above
22–30	·		+	·		+
30–38	−		·	·		+
38–44	−		·	·		−
44–50	+		or +	·		+
50–60	−		·	·		−
About 61	+		·	·		+

+ Above = contraction above base line.
− Above = relaxation but not below base line.
+ Below = contraction but not up to base line.
− Below = relaxation below base line.

The reactions to temperature changes do not depend upon the diameter of the arteries, large and small arteries giving precisely similar results.

The main point demonstrated by these experiments is that, within the viable range of rise in temperature, the systemic arteries relax while the coronary arteries contract; when excised from the body the coronary artery is completely relaxed, while the systemic artery is almost fully contracted. In the case of arteries kept for several hours without sufficient oxygen, the differences described become more pronounced. The results of these experiments should not be regarded as showing a fundamental difference between coronary and systemic arteries in general, for it is possible that systemic arteries other than those examined may show temperature reactions similar to those of the coronary arteries. The observations are important in that they provide an interpretation of the experiments on the effect of temperature upon the coronary circulation in the isolated heart and in the heart-lung preparation.

Action of adrenaline and ergotoxin. It is well known that in most animals adrenaline dilates the coronary and contracts the systemic arteries. We investigated the action of adrenaline upon these arteries after previous application of ergotoxin. The action of ergotoxin upon the peripheral blood vessels has been demonstrated by Dale(9) and by Barger and Dale(10), who showed that after the injection of ergotoxin, injection of adrenaline or stimulation of the splanchnic nerve causes a peripheral vaso-dilatation. This reversed effect of adrenaline was tentatively explained as being due to ergotoxin paralysing the sympathetic vaso-constrictor nerve endings, without, however, paralysing the sympathetic vaso-dilator nerve endings, and thus leaving the latter free to

respond to adrenaline or to stimulation of the sympathetic nerves. The vaso-dilator effect of adrenaline after ergotoxin depends upon the relative doses of the two drugs, and a large dose of adrenaline is known to overcome the effect of ergotoxin and so produce vaso-constriction. As regards isolated arterial rings, the effect of ergotoxin and the reversal phenomenon of Dale have not previously been investigated. In the coronary and systemic arteries we have vessels which behave oppositely towards adrenaline. If Dale's explanation of the reversal is correct we should expect to find that the vaso-dilator action of adrenaline upon the coronary arteries would remain unaffected by a previous application of ergotoxin in doses which in systemic arteries are sufficient to produce the reversal phenomenon.

Before settling this question it was necessary to determine whether a distinct reversed reaction to adrenaline can be observed on isolated systemic rings and whether ergotoxin by itself has any effect upon systemic and coronary rings.

(*a*) *Systemic arteries.* Ergotamine tartrate in concentrations below 1 : 500,000 at 37·5° C. failed to produce any effect, while concentrations between 1 : 500,000 to 1 : 100,000 produced a more or less conspicuous constriction which was followed by a slight dilatation or a return to the previous line. Still higher concentrations produced a pure dilatation which in many cases was so intense that the diameter of the artery was more than doubled. Ergotoxin added at room temperature even in the larger doses has no effect, but on warming, the systemic rings instead of their usual response relax progressively. This relaxation is very conspicuous and at 37·5° C. the rings may be doubled in diameter. In this relaxed state they continue to respond to barium chloride by contraction if the latter is added in large doses; after washing off the ergotoxin and cooling, they regain their tone and then show a normal temperature curve. As regards the action of adrenaline (tabloid form without chloreton) after the larger doses of ergotoxin we find that even large doses of adrenaline (up to 1 : 50,000) fail to have any effect. It is probable that in high concentration ergotoxin affects the muscle of the arteries, which relax, cease to react to temperature and react but slightly to vaso-constrictor substances. When ergotoxin is added in doses which, following the immediate constrictor effect, produce little or no vaso-dilatation, the addition of adrenaline either ceases to produce vaso-constriction, or causes dilatation. Whether ergotoxin abolishes or reverses the action of adrenaline depends upon the systemic artery employed. In the renal, carotid and the larger mesenteric arteries ergotoxin only abolishes the

effect of adrenaline; in small arteries it reverses the action of adrenaline. In this relation experiments upon the mesenteric arteries are instructive (Fig. 3). Curve *A* in Fig. 3 shows the response of a small mesenteric

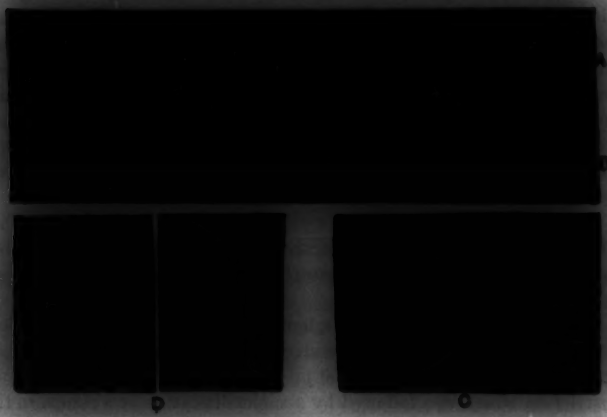

Fig. 3. All curves to be read from right to left.

Curve A. Small mesenteric arterial rings (1·5 mm. in diameter); at A adrenaline 1 : 1,000,000; at the apex of the contraction the rings are washed with about 100 c.c. of fluid; at E ergotamine tartrate 1 : 100,000, this is followed at A by a second dose of adrenaline 1 : 1,000,000; the rings are then again washed with about 100 c.c. of fluid.

Curve B. The same as curve *A* but larger mesenteric rings (4·0 mm. in diameter); adrenaline and ergotamine followed by another dose of adrenaline were added in the same concentrations as in curve *A*. The third dose of adrenaline (second after ergotamine) was 1 : 100,000; this was followed at B by barium chloride 1 : 15,000.

Curve C. Small mesenteric rings. Upper tracing—effect of adrenaline 1 : 800,000 before ergotamine. Lower tracing—same dose of adrenaline after ergotamine.

Curve D. Large mesenteric rings, same concentration of adrenaline as in curve *C*. Right-hand tracing before and left-hand tracing after ergotamine. All tracings were taken at a temperature of 37·5° C. Reduction $\frac{1}{3}$.

artery which was taken close to the intestine; curve *B* is that of the same mesenteric artery but taken at the root of the mesentery. In both cases the first administration of adrenaline gave a definite constrictor effect. The Ringer's fluid in the Dewar's flask was then changed and time was allowed for the rings to relax. Administration of ergotoxin

was followed in both cases by a transitory constriction after which the arteries relaxed to slightly below their respective base lines. A second administration of adrenaline caused a conspicuous dilatation of the smaller rings but had no effect upon the larger ones. In the latter case even a considerably larger dose of adrenaline which was administered next also failed to have any effect. The smaller rings returned after washing to their original base line; the larger rings though they did not respond to adrenaline constricted on administration of barium chloride. Curve *C* shows the response to adrenaline of a small mesenteric artery before and after ergotoxin; curve *D* shows a response of a large artery. Similar results were obtained in every experiment in which the reversal phenomenon was studied on rings taken from the main trunks of different arteries and from their smaller branches.

On the basis of these experiments we may conclude that isolated arterial rings are suitable for the study of the reversal phenomenon. If the reversal is due to paralysis of the sympathetic vaso-constrictors then we have to assume that only the smaller ramifications of the arteries are supplied with sympathetic vaso-dilators.

(*b*) *Coronary arteries.* Small doses of ergotoxin (1 : 500,000–1 : 100,000) added at 37·5° C. produce either no effect or a transitory vaso-constriction, followed by a vaso-dilatation, the extent of which depends on the dose. In either case adrenaline continues to produce a marked vaso-dilatation. This was observed even when ergotoxin was added in doses considerably larger than those sufficient to abolish or reverse the effect of adrenaline upon systemic rings. Fig. 4 shows curves obtained from a series of experiments upon the same three rings of a coronary artery. Curve *A* is a normal temperature reaction with the vaso-dilator response to a dose of 1 : 800,000 of adrenaline at 37·5° C. The arterial rings were thoroughly washed in Ringer's solution at 37·5° C. and cooled to 15° C. The temperature was again raised to 37·5° C., and ergotoxin, 1 : 50,000 added (curve *B*). After a transient contraction, a definite relaxation occurred; after which adrenaline (1 : 800,000) added to the perfusion fluid produced a complete relaxation, the curve nearly returning to its original base line. The arterial rings were now thoroughly washed with warm Ringer's solution of 37·5° C. After the original tone was regained ergotoxin was added in two doses, giving a final concentration of 1 : 37,500. The degree of relaxation was comparable to that in the previous experiment; adrenaline was then added to a concentration of 1 : 800,000, the completion of the curve of relaxation being again comparable to the preceding one. In no case have we found

ergotoxin plus adrenaline to relax the coronary artery below its original base line at room temperature. Curve *D* shows the temperature reaction

Fig. 4. All curves to be read from right to left.

Curve A. Normal temperature curve of fresh coronary rings. At A adrenaline 1 : 800,000 was added; the rings were then washed with cold fluid. The effect of re-warming is shown by curve *B*.

Curve B. Ergotamine 1 : 50,000 was added at E and adrenaline same dose as before, at A. The rings after thorough washing with warm fluid spontaneously regain their tone (curve *C*).

Curve C. Ergotamine is added in two doses making a final concentration of 1 : 37,500; adrenaline 1 : 800,000 again relaxes the rings. The rings are thoroughly washed with cold fluid and ergotamine 1 : 50,000 is added before warming.

Curve D. Shows the effect of warming in presence of ergotamine. At E another dose of ergotamine was added making the final concentration 1 : 37,500; adrenaline 1 : 800,000 added at A relaxes the rings to the original base line. The rings are thoroughly washed in a stream of warm fluid.

Curve E. Shows the recovery of the rings on washing.

All the curves were obtained from the same rings. Rate of drum 1 mm. per minute. Reduction ½.

in the presence of ergotoxin (1 : 50,000), and curve *E* the recovery of the artery on washing. It is thus seen that in large doses ergotoxin antagonises the temperature response of the coronary rings. From a comparison of curves obtained from rings taken from the coronary artery at its origin, and from the smaller arteries within the muscle at the heart's apex, it can be seen that the small coronary vessels are proportionally more reactive to adrenaline than the larger; this would

indicate that the smaller the vessel and the finer its ramification, the richer is it in vaso-dilator supply.

THE EFFECT OF HISTAMINE ON CORONARY ARTERIES. We have found that in the coronary and systemic arterial rings of the dog, man and the ox histamine always caused contraction (Fig. 5). In perfusion experiments on non-beating strips of the ventricular muscle of the rabbit's heart, histamine produced diminution in the rate of perfusion. In experiments with perfusion of long pieces of coronary arteries taken from the heart of the rabbit, histamine also caused contraction. The doses of histamine used varied in the case of perfusion experiments from 1 in 10,000,000 to 1 in 1,000,000, the H-ion concentration of the perfusing fluid being kept constant. The contraction, whether produced on perfused strips of ventricle, perfused pieces of arteries or arterial rings, could be effectively abolished by adrenaline. This forms a very convenient method of showing that in the rabbit and dog adrenaline has a definite vaso-dilator effect, which is independent of the changes that it may induce in the heart muscle.

Fig. 5. Effect of histamine (1 : 200,000) upon the coronary rings of the ox; temperature 37·5° C.; three rings are used in a chain. The inside diameter of each ring was at room temperature 2·3 mm., at 37·5° C. 1·5 mm., after addition of histamine 0·5 mm. The effect of histamine was antagonised by addition of adrenaline (1 : 400,000). Reduction ⅓.

Experiment. Strip of rabbit's ventricle perfused through left coronary artery with non-oxygenated Ringer's fluid at a pressure of 80 cm. of water; temperature 36·5° C., *p*H 7·4.

Drops per minute: 43, 44, 43, 44, 44. Perfusion changed to Ringer's with 1 : 1,000,000 histamine: 43, 31, 23, 17, 16, 13, 11, 12, 12, 10, 12, 11, 12. Injection into cannula of 0·1 c.c. adrenaline 1 : 1,000,000, 12, 16, 20, 21, 17, 14, 12, 11, 10, 11, 10, 11. Injection of 0·1 c.c. adrenaline 1 : 100,000, 27, 34, 36, 35, 22, 17, 15, 14, 15, 11, 13, 10, 11, 10.

The smallest doses of adrenaline which under these conditions were active always produced vaso-dilatation. We failed to observe the pre-

liminary vaso-constriction described by Brodie and Cullis(11) in their experiments upon the perfused beating heart.

In the absence of oxygen the ventricular strips did not contract nor did they exhibit the tonic contracture described by Hammouda and Kinosita(12). The increased rate of perfusion was therefore due purely to vaso-dilatation. The same results were observed in perfusion of pieces of rabbits' coronary arteries and upon rings of coronary arteries of the ox and dog (Fig. 5).

In the cat histamine produces vaso-dilatation of the perfused strips which corroborates the experiments of Gunn(13).

Experiment. Strip of cat's ventricle; conditions same as in above experiment.

Drops per minute: 36, 35, 35, 35. Histamine, Ringer 1 : 2,500,000: 37, 44, 52, 54, 56, 56, 56, 57. Normal Ringer: 57, 56, 55, 49; after 5 minutes 36, 35, 35.

Conclusions.

1. Freshly excised systemic arteries of ox, dog and man (carotid, renal, mesenteric and femoral) are at room temperature contracted, while the coronary arteries are completely relaxed.

With a temperature rising from 27° C. to 38° C. the systemic and coronary arteries behave differently: the systemic arteries relax while the coronaries contract.

2. With a temperature rising from 44° C. to 60° C. the coronary and systemic arteries behave similarly, showing a heat paralysis at 48°–50° C. and a heat contracture at 60°–61° C.

3. Isolated systemic rings are suitable for the study of the reversed effect of adrenaline after administration of ergotoxin. The reversal phenomenon is observed only on the smaller ramifications of arteries; in the larger arteries ergotoxin abolishes the constrictor effect of adrenaline but fails to produce a reversal.

4. Ergotoxin does not abolish the dialator effect of adrenaline upon coronary arteries.

5. Histamine contracts the coronary arteries of the ox, rabbit and man and dilates the blood vessels of a perfused strip of cat's ventricle.

We wish to record our thanks to Dr G. V. Anrep for the suggestion of the problem and for his continued interest and help throughout the experiments.

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THE PRESSURE EQUILIBRIUM OF THE EYE.

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In a previous series of papers(1) it was found that the equilibrium of the physical forces in the eye—the difference between the intra-ocular pressure and the blood-pressures, and the difference between the osmotic pressures of the aqueous humour and the plasma—showed nothing incompatible with the hypothesis that the intra-ocular fluids were a dialysate of the capillary blood. It was further shown that in its chemical composition(2) the aqueous humour was in equilibrium with the plasma, since it contained only traces of all the colloidal constituents of this fluid, while the non-dissociated crystalloids were represented in equal amounts in the two, and the ionised constituents were partitioned between them in the concentrations necessitated by a system in membrane equilibrium. It was also found that the abnormal aqueous humour formed either by increasing the permeability of the capillary walls or by altering the concentration of the diffusible constituents of the blood, maintained the same relationship. Finally, it was suggested(3) that the circulation of the intra-ocular fluids as commonly conceived had been determined and measured only by experiments wherein the normal pressure conditions had been upset by opening the eye, or by making injections into it, or by employing other procedures which could be interpreted as calculated to initiate artificially an abnormal current. On the other hand, while there was a metabolic interchange between the aqueous humour and the plasma through the capillary walls throughout the vascularised tissues of the eye, consisting of a free diffusion of crystalloids in both directions and a re-adjustment depending on colloidal impermeabilities, and also an "internal" thermal circulation in the anterior chamber made up of convection currents determined by the difference in temperature (3°–5° C.) between the vascularised iris and the air-cooled cornea, it was pointed out that the evidence indicated that the minimal circulation which the intra-ocular fluids possessed

seemed to be impressed upon it secondarily by constantly occurring changes of pressure due largely to contractions of the internal and external musculature of the eye. In this case the aqueous humour is strictly comparable to the tissue-fluids of the body generally. The only difference, in fact, between the two seems to depend on the relative impermeability of the capillaries of the eye to colloidal micelles, presumably a biological adaptation to render the intra-ocular fluids an optically homogeneous medium.

If the aqueous humour is a dialysate, the level of the intra-ocular pressure is determined by the height of the hydrostatic pressure in the capillaries of the eye minus the difference between the osmotic pressure of the capillary blood and the aqueous: this we have seen(1) to be in accordance with experimental findings. Further the variations in the intra-ocular pressure should be determined by alterations in either of these two factors. At the beginning of this century Parsons(4) and Henderson and Starling(5) showed that the intra-ocular pressure varies very intimately with the blood-pressure, and they concluded that there was evidence of no other regulating mechanism. The fact that under clinical conditions the two could not by any means be so closely correlated, has provided one of the strongest arguments in favour of a secretory origin of the intra-ocular fluids. These investigators, however, worked under conditions wherein the osmotic pressure of the blood and the aqueous remained unchanged. The following experiments were undertaken to supplement their work, and to determine the influence of this latter factor in inducing variations in the pressure equilibrium of the eye.

Experimental.

The animals used throughout were cats. Anæsthesia was induced with ether and maintained by intravenous chloralose. The blood-pressure was registered by a mercury manometer connected with a cannula inserted into the carotid artery. The intra-ocular pressure was taken by means of a compensated mercury manometer, an air-bubble in a horizontal capillary tube being employed as an index of equilibrium as was used by Henderson and Starling(5).

1. As a control a tracing (Fig. 1) is reproduced showing the intimate manner in which the intra-ocular pressure varies with the blood-pressure, the osmotic conditions throughout remaining unchanged. The blood-pressure was varied by ligating the abdominal aorta, releasing the ligature suddenly, tightening it again and slackening it slowly. The curve of the intra-ocular pressure is seen to follow roughly the upward

rise of the blood-pressure in steps and to fall coincidently with it to its original level. The sudden fall in blood-pressure between *b* and *c* is not

Fig. 1. The variation of the intra-ocular pressure with the blood-pressure.

The upper tracing the blood-pressure; the lower the intra-ocular pressure. At (*a*) the abdominal aorta ligated; at (*b*) the ligature released suddenly; at (*c*) the abdominal aorta again ligated; at (*d*) the ligature released slowly. The intra-ocular pressure follows approximately the blood-pressure. (Cat.)

accompanied by a corresponding fall in the intra-ocular pressure, presumably owing to its short duration and the inertia of the manometer employed.

2. A series of experiments were done in which the blood-pressure and the composition of the blood were both altered without, however, changing the osmotic equilibrium. The femoral artery was opened and allowed to bleed until the blood-pressure had fallen to about one-half of its original height (Fig. 2); the intra-ocular pressure followed suit. The volume of blood was then made up by the intravenous injection of an osmotically equivalent solution of gum arabic in Ringer until the blood-pressure had reached its original height: the intra-ocular pressure rose coincidently and remained indefinitely at the corresponding level.

3. A series of experiments were done in which the fluid volume after bleeding was made up by transfusion with a concentrated gum arabic solution. Fig. 3 represents the effect of a 15 p.c. gum arabic solution in tap water. The curve of the intra-ocular pressure followed the blood-pressure closely at first; but soon after the injection it began to fall steadily, independently of the latter. By this procedure the colloid content of the plasma was greatly increased. Meantime refractive

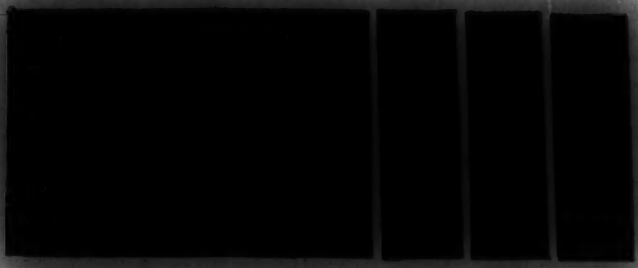

Fig. 2. The variation of the intra-ocular pressure on altering the blood-pressure and the composition of the blood, the osmotic conditions remaining constant.

The upper tracing the blood-pressure; the lower the intra-ocular pressure. At (*a*) the femoral artery opened; at (*b*) the bleeding stopped and injection of physiological saline and gum arabic commenced, and continued until (*c*). The intra-ocular pressure follows approximately the blood-pressure. (Cat.)

Fig. 3. Variation of the intra-ocular pressure on increasing the colloid osmotic pressure of the blood.

The upper curve the blood-pressure; the lower the intra-ocular pressure. At (*a*) the femoral artery opened; at (*b*) the bleeding stopped; at (*c*) injection of 15 p.c. gum arabic; at (*d*) injection stopped. The intra-ocular pressure, after following the blood-pressure initially, tends to fall. (Cat.)

estimations carried out on the aqueous of the other eye showed that no colloidal molecules had passed the capillary walls. The difference in the osmotic pressure between the two, which depends upon their relative colloid content, was therefore necessarily increased, and the intra-ocular pressure, whose level is the capillary pressure less this difference, consequently fell.

Such a fall of pressure persists over a considerable time. A rabbit was injected with a similar solution (30 c.c. into the marginal vein of the ear), and the tension was taken in the intact eye by means of a Schiötz tonometer, cocaine being used as a local anæsthetic. A typical curve is shown in Fig. 4, which shows the marked and prolonged fall in

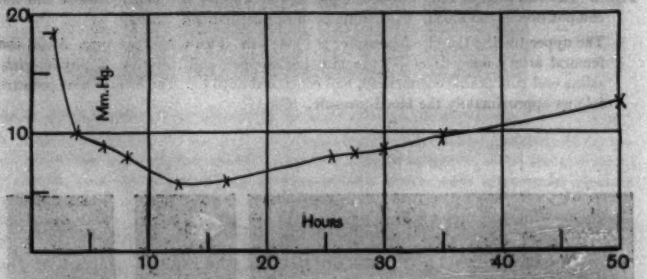

Fig. 4. Variation of the intra-ocular pressure on increasing the colloid osmotic pressure of the blood.

30 c.c. 15 p.c. gum arabic injected intravenously at second hour. Curve of intra-ocular pressure read on a Schiötz tonometer (cocaine) and transposed into mm. Hg. The crosses denote the times of reading. (Rabbit.)

the ocular tension. The injection was done without previous bleeding, and although simultaneous blood-pressures could not be recorded owing to the length of the experiment it is almost certain that this would have risen to some extent.

4. A cat was similarly bled, and the fluid volume was made up by the intravenous injection of physiological saline until the original blood-pressure was reached and maintained (Fig. 5). The intra-ocular pressure at first followed the blood-pressure and then, dissociating itself from it, rose steadily. By this procedure the blood colloids were diluted, and since the saline diffused freely into the eye and the osmotic pressure of the diffusible constituents of the two fluids (blood and aqueous) quickly attained equilibrium(2), the difference between the total osmotic pressures

was diminished, and the intra-ocular pressure consequently rose to approach the level of the hydrostatic pressure in the capillaries.

Fig. 5. Variation of the intra-ocular pressure on decreasing the colloid osmotic pressure of the blood.

The upper curve the blood-pressure; the lower the intra-ocular pressure. At (*a*) the femoral artery opened; at (*b*) the artery closed and physiological saline slowly injected; at (*c*) the saline injected rapidly. The intra-ocular pressure, after following the blood-pressure initially, tends to rise. (Cat.)

5. A similar change occurs on varying the colloid content of the aqueous humour. The tracing of a typical experiment showing this is

Fig. 6. Variation of the intra-ocular pressure on altering the composition of the aqueous humour.

The upper curve the blood-pressure; the lower the intra-ocular pressure. During (*ab*) the aqueous humour replaced by physiological saline; during (*cd*) the aqueous humour replaced by 15 p.c. gum arabic. In the first case the intra-ocular pressure remains approximately constant; in the second it tends to rise. (Cat.)

illustrated in Fig. 6. Two needles were introduced into the anterior chamber of a cat each of which was connected with a reservoir, the one

containing physiological saline and the other a 15 p.c. solution of gum arabic in tap water. The former was connected with the eye by means of a calibrated tube containing an air-bubble, and communication with the latter was prevented at first by means of a stop-cock. The saline reservoir was kept at the level of the normal intra-ocular pressure for some time after the initial disturbance subsequent to the insertion of the needles had subsided: that this was so was made evident by there being no movement of the air-bubble. After equilibrium had been established for some time the saline reservoir was raised to a point 1 cm. above this level, and the gum reservoir was lowered a similar amount and its connection with the eye opened. In this way a slow flow of saline was initiated from the saline reservoir through the eye into the gum reservoir. When the air-bubble travelling along the calibrated tube with this current showed that 0·3 c.c. of saline had entered the eye, the gum reservoir was clamped off, and the saline reservoir was lowered again to the height of the intra-ocular pressure as was demonstrated by the movement of the air-bubble ceasing. It is seen from the tracing that the intra-ocular pressure remained substantially as before. The eye of the cat contains about 0·25 c.c. aqueous, and it may be taken that this fluid had been replaced to a considerable extent by saline, but since the normal aqueous of the cat contains only 0·02 p.c. protein (3), the osmotic conditions are little upset. The clamp was then taken off the gum reservoir, and a flow in the opposite direction was induced by manipulating the heights of the two reservoirs in the opposite way until the air-bubble showed the passage of 0·9 c.c. of fluid. At this point the eye would contain a considerable concentration of colloid. The gum reservoir was then shut off, and the intra-ocular pressure was observed in the previous manner. The tracing shows that it rose steadily, an effect presumably due to the colloid osmotic pressure of the intra-ocular fluids approximating that of the plasma, and therefore causing the intra-ocular pressure to rise towards the level of the hydrostatic pressure in the capillaries. As was to be expected, a simultaneous blood-pressure tracing shows no evidence of change.

Discussion.

The intra-ocular pressure is maintained at its normal level by the balance between the hydrostatic pressure in the capillaries and the difference between the osmotic pressures of the aqueous humour and the capillary plasma. Being maintained in equilibrium at this level, it

may be varied by several factors other than changes in the blood-pressure, which have hitherto been generally accepted as the only determining variant.

The intra-ocular pressure may be varied:

1. By altering the equilibrium level:

(*a*) By raising or lowering the blood-pressure in the capillaries. This may be effected either by varying the general blood-pressure or by altering the height of the capillary pressure locally by physical, chemical, nervous, hormonal, or psychical influences.

(*b*) By varying the difference between the osmotic pressure of the capillary plasma and the aqueous humour. Since the capillaries are freely permeable to crystalloids, this is attained by increasing or decreasing the concentration of colloids in the former, or by increasing the colloid content of the latter.

2. By altering the volume pressure inside the eye. This influence is rendered possible because of the small distensibility of the sclerotic which at once translates any increase of volume into an increase of pressure.

(*a*) By dilating or contracting the capillaries, a factor subject to the same influences as variations in the pressure of the capillaries.

(*b*) By increasing or diminishing the volume of the aqueous humour. In this way hypotony can be produced by squeezing out some of the aqueous by the application of external pressure to the globe, a circumstance which is taken advantage of clinically in massage of the eye in states of raised tension. The volume of this fluid may also be either increased or decreased in the osmotic hydræmia or depletion of fluid which follows the intravenous injection of large quantities of hypotonic or hypertonic saline; I have shown (6) that these respectively bring about an increase or decrease of the intra-ocular pressure independently of the blood-pressure. In these experiments it was demonstrated by a gravimetric estimation of the water-content of one eye before the injection and of the other afterwards, that the changes of pressure depended in large measure upon changes in the quantity of intra-ocular fluid, although the prolonged nature of the pressure variations suggested that physico-chemical changes in the vitreous were also to some extent responsible.

(*b*) By increasing or diminishing the volume of the lens and vitreous, the turgidity pressure of which changes markedly with alterations in the concentration of hydrogen ions and salts in the fluids bathing them. This is probably an important factor in the changes of intra-ocular pressure met with pathologically. Some preliminary experiments in-

vestigating these reactions have been carried out, and it is hoped to amplify these in the near future.

It was seen(1) that in the normal state the pressure in the venous exits was slightly in excess of the intra-ocular pressure (1 to 2 mm. Hg), but that when the volume pressure of the eye was increased, the intra-ocular pressure temporarily rose above that in the veins. These therefore, and of them most especially the canal of Schlemm, act as a safety valve mechanism which in normal circumstances deals adequately with the "pressure circulation" of the aqueous humour, and to some extent counteracts the changes of volume pressure which may occur in the eye; but when they are obliterated or their efficiency is lessened, this safety valve action is lost, and physiological and pathological rises in tension will tend to be cumulative and their effects to be permanent.

Conclusions.

It has hitherto been generally accepted that the intra-ocular pressure varies with the hydrostatic blood-pressure and with it alone.

Experiments are described confirming this relationship, and showing that the intra-ocular pressure varies also with the difference between the osmotic pressure of the blood and the aqueous humour. The influence of other variants is pointed out, which act by altering the volume-pressure of the eye.

So far as these experiments go, they are in agreement with the hypothesis that the aqueous humour is a dialysate of the capillary plasma; and it is suggested that they may help to indicate a direction along which seemingly inexplicable clinical variations in the intra-ocular tension may be explained by physiological experiment.

I was greatly indebted to the late Prof. Starling for his encouragement and interest in carrying out these experiments.

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OBSERVATIONS UPON A PILOMOTOR REACTION IN RESPONSE TO FARADISM[1].

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In observations upon vascular reactions of the human skin we have frequently noted that, when the skin is stimulated by means of a strong faradic current, goose skin appears in the area surrounding the electrodes. The area involved in the reaction is sometimes so large as seemingly to preclude the possibility of direct excitation of the pilomotor muscles, or of the nerve endings immediately supplying these, by spread of current; the extensiveness of the reaction first suggested to us the possibility of a local spread of impulses through the pilomotor nerves and we therefore determined to investigate the reaction more closely.

To avoid possible misunderstandings, it may be well to state at this stage that stimuli applied to the human skin are capable of causing an erection of the hairs in one of three distinct ways. The first and second of these are already known, the third is that which we shall particularly describe.

(1) *Central reflex.* If the body is unclothed in a cool room and one of a variety of appropriate stimuli is applied to certain areas, goose skin appears and spreads over a large surface of the body; it may completely involve one side of the body or even the whole body. Friction, the application of hot or cold water, or of electrical currents, form adequate stimuli; and it is characteristic that stimuli to be effective are usually weak stimuli applied to a relatively large surface. It is also to be remarked that the response is obtained most readily from certain regions, such as the neck and the front part of the chest. The latent period is brief and the goose skin reaction spreads over the body in a wave-like fashion, the erection quickly subsiding at a given point.

This type of reaction was first studied by Mackenzie(1) (see also Trotter and Davies(2), and Sobotka(3)), and it is due to a reflex through the central nervous system.

[1] Work undertaken on behalf of the Medical Research Council.

[2] Fellow of the John Simon Guggenheim Memorial Foundation.

(2) *Local response.* If the skin is heavily stroked, or if ice is laid upon it for some minutes, goose skin appears over an area corresponding to that stimulated. This local reaction occurs after the cutaneous nerves have been divided and have been allowed to degenerate. Thus, Trotter and Davies speak of the reaction to strokes remaining intact after nerve section, and Mr Trotter tells us that in these observations the reaction persisted until after nerve degeneration had had time to occur. So far as the application of ice is concerned we make this statement from personal observations upon three cases, in which this stimulus was used 32, 54 and 55 days after surgical division of a cutaneous nerve. The areas tested were totally insensitive to touch, pain, heat and cold. Unmistakable goose skin appeared in the cooled insensitive skin, though it was somewhat less prominent than that appearing when a symmetrical area of normal skin was similarly cooled.

The integrity of the local pilomotor mechanism after nerve section and degeneration is shown by these observations and by an exaggerated response of this mechanism to adrenalin in the cat and in man (Elliott(4), Trotter and Davies(2)). It is clear that heavy stroking and the application of cold can cause erection of the hairs by directly stimulating this local mechanism. The erection is comparatively long lasting.

(3) *Local spread.* Heavy stroking, though it usually produces a reaction strictly limited to the line of the strokes, may yield in addition a narrow band of goose skin on either side of this line(1, 3). This marginal reaction has been regarded also as a direct effect, the supposition being that the mechanical stimulus is not confined to the actual line of contact (Sobotka(3), p. 517).

Similarly, when a faradic current is applied to the skin and yields an area of goose skin extending some distance beyond the actual area of electrode contact, it has been assumed that this marginal goose skin is due to spread of the faradic current into the skin for a corresponding distance, and to direct stimulation of the muscular elements in the skin (Sobotka(3), Koenigsfeld and Zierl(5)).

In neither instance, however, has evidence been adduced to prove or disprove the view that the marginal reaction is the result of direct stimulation; the argument rests upon a physical and not upon a physiological basis.

These preliminary remarks will suffice and we may proceed to examine in detail the local reaction to the more convenient form of stimulation, namely the faradic current.

The local reaction to faradism.

The local faradic reaction can be obtained on the scalp, face, trunk and limbs. We have studied it in its details on the skin of arm and leg. It has been described and discussed briefly by Sobotka previously.

A du Bois Reymond coil is connected to a 2 volt accumulator, and the current from the secondary coil carried to twin platinum contacts (lying 1½ mm. apart). The secondary coil is placed at such a distance that, when the current is led into the unmoistened skin it is quite painful, in fact almost intolerable. A faradic current of this intensity is usually necessary to give full elevation of the hairs. In about 4 or 5 seconds the skin begins to show small discrete elevations, best seen when these are obliquely illuminated; they quickly grow in prominence until full goose skin is manifested in about 10 or 20 seconds. Upon the forearm the area at first involved extends a centimetre or two from the electrodes in every direction, but, if the current is maintained, it spreads farther in the skin, until it may involve an area approximately circular and 5 to 6 or more cm. in diameter (Fig. 1). On the leg the area is often larger.

When the current is maintained for 10 or 20 seconds the area of goose skin for some distance around the electrodes is compact, that is to say every papilla, or almost every papilla, in it is erected. The erection is also full over this area. Marginally the reaction does not involve every papilla, and some or many are erected imperfectly; in these respects there are variations. Parts of the margin may show full erection of the hairs, so that there is an abrupt transition from compact and full goose skin to skin in which no reaction is seen. In other parts the transition is less abrupt; here however the transition occurs over a relatively narrow zone of skin, usually no more than a few papillæ deep. Marginally, compactness may fail where the papillæ are fully, or where they are only partially, erected. Uniform decline in the height of the erected papillæ, when these are traced from the point stimulated to the margin of the reaction, is not seen.

If the electrodes are moved a little distance, in general a new band of goose skin is added on the side to which movement occurs and is subtracted from the side from which they move. Thus the electrodes continue to lie at or near the centre of the corresponding reaction. Over the portion of skin common to both reactions the same hairs are erected, for in each case a compact area of goose skin surrounds the

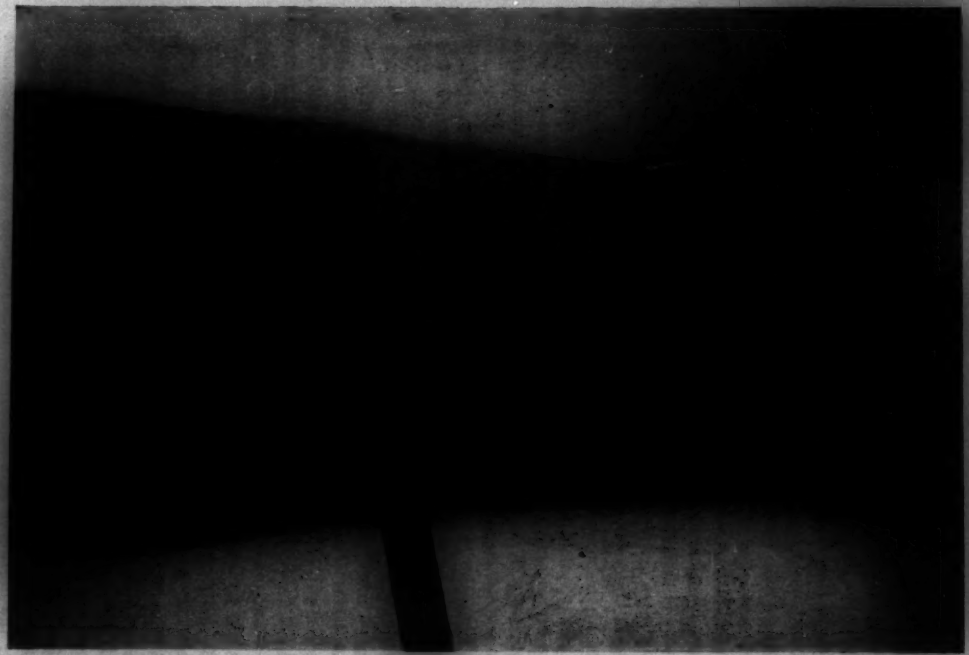

Fig. 1. Reaction of the skin upon the dorsal surface of the forearm to a strong faradic current. The outermost papillæ to be erected are marked by the letters *a* and *b*.

point stimulated. Thus any given hair may be erected by stimulating the skin within 1 or 2 cm. distance of it in any direction.

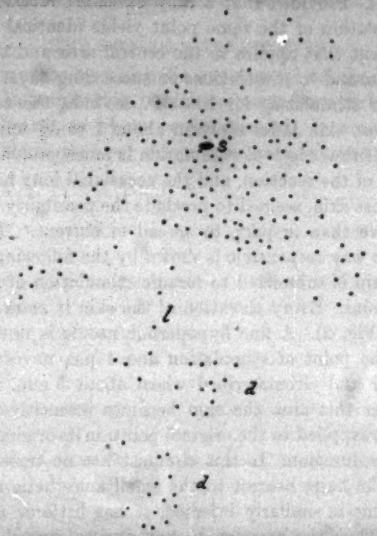

Fig. 2. (×¾.) The skin of the leg was strongly stimulated faradically at a point *s*; the current being continued for a number of minutes. The resultant goose skin was mapped out by spotting each erected papilla with indian ink. This diagram is a tracing subsequently made from the skin. In addition to a compact area of goose skin around *s*, it shows outlying projections, a line of six erected hairs at *l* and two islets of goose skin at *d*, *d*. Here and subsequently, the bottom of the diagram represents the distal portion of the reaction on the limb.

Now the area of the full reaction is never truly circular and, particularly if the current is maintained so that the area enlarges, there are promontories of lesser or greater extent jutting out from the main area involved. Not infrequently detached islets of goose skin occur (*d*, Figs. 2 and 3). These promontories and islets vary in their constitution, often the erection is less complete in these, sometimes it is full; the papillæ erected in the islet may be scattered; more frequently they are erected compactly or almost so. The promontory or outlying islet is usually elongated in form, in which case its long axis lies more or less radially to the point stimulated; curious radiating lines of goose

The outermost papillæ to be erected are marked by the letters *a* and *b*.

skin (*l*, Figs. 2 and 3) occasionally appear. The greatest extension of goose skin from the point stimulated that we have seen has been 10 cm. (Figs. 2 and 3). Provided that a fully extended reaction is obtained, repeated stimulation of the same point yields identical areas of goose skin, a statement that applies to the central area and to the promontories and islets, and to stimulations on succeeding days.

When, after stimulating for 10 or 20 seconds, the current is withdrawn, the goose skin fades away in about 1 to 1½ minutes; it tends to last longer, if the length of stimulation is much prolonged.

The extent of the reaction, and the occasional long tongues or islets of marginal goose skin, seemed to preclude the possibility of the reaction being due, more than in part, to spread of current. That spread of current is in no way responsible is shown by the following simple observation. The skin is submitted to faradic stimulation of given strength at a marked point. Every elevation of the skin is marked on the skin surface (as in Fig. 2). A fine hypodermic needle is now run into the true skin at the point of stimulation and 1 p.c. novocain is injected until a circular and circumscribed wheal about 5 mm. in diameter is produced. Over this area the skin becomes insensitive. The faradic current is now reapplied to the original point, in its original strength and for its previous duration. In this circumstance no trace of goose skin is seen; even the hairs nearest to the small anæsthetic area fail to be erected. If saline is similarly injected, it has little or no effect upon the reaction. Thus the reaction is not due to spread of current, a conclusion fully confirmed by other observations subsequently to be described. It is due to stimulation of cutaneous nerves at the point to which the current is applied and to spread of impulses through these. In harmony with this conclusion is the following observation. A cutaneous nerve was cut surgically on the forearm of one subject and the goose skin reactions tested 54 days later, an interval amply sufficient to allow of nerve degeneration. A cold bath was followed by general goose skin, the anæsthetic area alone failing to react; a very strong faradic current repeatedly applied to the anæsthetic area failed to produce a reaction; a weaker current gave distinct goose skin upon the control skin of the other arm. A flat piece of ice laid upon the control skin gave prominent local goose skin; laid upon the anæsthetic area the reaction was distinct though somewhat less prominent and delayed in its appearance. Similarly direct stimulation, by striking the skin smartly with a flat stick, poduced goose skin over the anæsthetic area. The last observations are important in showing that in the area to which

nerve degeneration had occurred, and which failed to react to faradism, the muscular part of the pilomotor mechanism was intact and capable of responding to suitable stimulation.

The nervous paths involved.

The nervous paths by which the impulses spread can be investigated a little further in human skin. In these observations we have been aided by Dr R. T. Grant, to whom we are grateful. In a number of observations we have mapped out the precise areas of goose skin associated

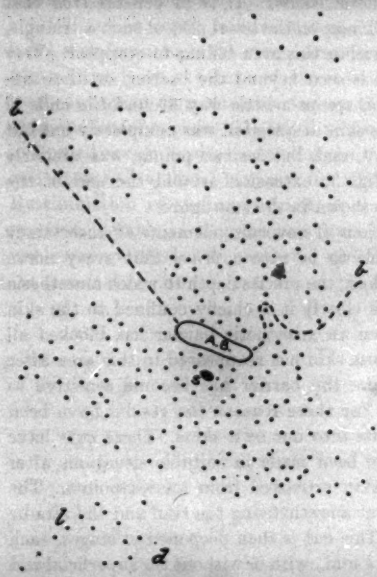

Fig. 3.

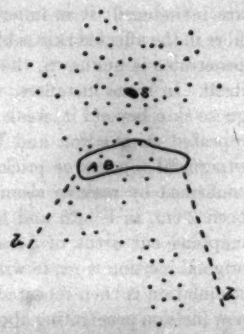

Fig. 4.

Fig. 3. (×¾.) A similar diagram of goose skin excited from point *s* on the leg. After mapping out this goose skin, an anæsthetic barrier was introduced and is outlined by *A.B.* On restimulating the point *s*, the same hairs were erected below the barrier and the lines *b*, *b*, but those above failed to appear.

Fig. 4. (×¾.) A small area of goose skin produced by stimulating the leg at *s*. The anæsthetic area *A.B.*, subsequently laid down, prevented re-erection of the hairs beyond the barrier and the lines *b*, *b*. The area of goose skin around *s* reappeared regularly with restimulation.

with given points of faradic stimulation and have proceeded to lay down anæsthetic barriers in the skin by means of novocain. These barriers have been usually about 1·5 to 2 cm. long, and are laid down by intradermal injection, the needle being advanced in steps along a straight line as the injection is made. The barrier is laid down tangentially and usually about 1 cm. from the point of stimulation (Figs. 3 and 4). If imaginary lines are now drawn from the stimulated point through the ends of the anæsthetic barrier, a triangular area of skin is mapped out, the apical parts of which lie on the near side of, and the basal parts of which lie beyond, the anæsthetic barrier. It is in general true that restimulation yields no goose skin upon the basal part of such a triangle, the original elevations lying within this area failing to reappear. Very exceptionally a weak reaction is seen beyond the barrier, or more frequently the goose skin reaction creeps a little way around the ends of the barrier. The area of goose skin in question was completely cut out in the example shown in Fig. 4, and, but for two points, was similarly affected in the example of Fig. 3; extension around the end of the barrier may be greater than is shown in the last figure.

In using intradermal injections of novocain, elements of uncertainty are introduced; it is impossible to be sure *a priori* that every nerve fibre in the affected skin is blocked; the precise depth to which anæsthesia penetrates is unknown, though clearly it is chiefly confined to the skin itself. In some instances, when an anæsthetic barrier has blocked all goose skin beyond it, weak goose skin has reappeared in this area after repeated stimulation and before the barrier has become sensitive to superficial touches or pricks. For these reasons the results have been confirmed by making clean cuts into our own skins. These cuts have been 2 cm. in length and have been made in suitable situations after mapping out areas of goose skin provoked from known points. The original incision is made without anæsthetising the skin and the faradic stimulation is then repeated. This cut is then deepened in stages, each new incision penetrating about $\frac{1}{2}$ mm., with or without the superficial and momentary application of a little 1 p.c. novocain, until the goose skin reaction on the side of the incision away from stimulation is weakened or is lost; about six incisions, each $\frac{1}{2}$ mm. deep, suffice. At each stage of the experiment careful measurements of the depth and gape of the wound are made and the tissue lying at the base of the wound is closely inspected. In the margin of cut skin the subpapillary vascular plexus is easily recognisable, showing as a thin red line. The first incision penetrates this layer throughout and cuts more deeply into the true

skin; but it is necessary to deepen the incision to 3 or 4 mm. before the goose skin reaction to faradism is affected. Thus in one instance in which the cut penetrated 3·3 mm. into the skin, fat was clearly seen in the base of the wound, but it was intersected by small whitish strands of the deeper layers of the true skin. The goose skin reaction was unaffected at the time, but was seen to be notably weakened on the side of the incision away from stimulation on a few succeeding days, after which full goose skin became restored[1]. This cut was just insufficiently deep to sever all channels; those remaining were presumably affected for a few days by the inflammatory reaction. In another subject a cut 3 mm. in depth just reached pure fat along its whole length; the last $\frac{1}{2}$ mm. incision, as the wound was deepened, divided small remaining white strands at the base of the wound. Before this last incision, a weakened goose skin was observed on the side away from stimulation; after the incision, goose skin failed to appear beyond the wound, except in a small area opposite one end of the incision. The wound at this end was deepened a little, but without affecting the reaction as a whole. Restimulation of the same point on the next day gave an identical reaction, namely, full goose skin up to the incision, a small area of weakened goose skin beyond one end of the incision, but none over

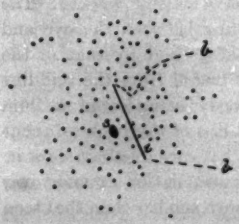

Fig. 5.

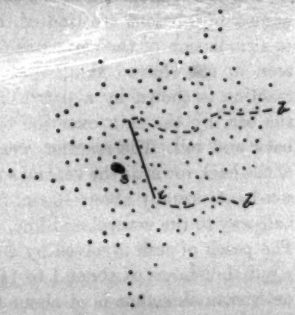

Fig. 6.

Fig. 5. ($\times\frac{1}{2}$.) An area of goose skin produced on the upper arm by faradising point *s*. The erection of the hairs lying between lines *b*, *b* was prevented by an incision *i* just penetrating through the true skin.

Fig. 6. ($\times\frac{1}{2}$.) A tracing of a similar experiment upon another subject.

[1] The edges of such cuts were brought into close apposition by means of horse hair stitches; the cuts healed promptly.

the greater part of the area originally presenting it beyond the wound (Fig. 5, see also Fig. 6 from a third subject). This modified reaction was obtained in exactly the same form on many succeeding days (tested up to the 34th day).

When an incision forms a barrier to the passage of goose skin in one direction, it forms an equal barrier to conduction of the reaction in the reverse direction; in both tests, goose skin appears fully on the stimulated side right up to but not beyond the actual incision, unless there is a little spread around the ends of the incision. Stimulation at the end of the incision yields full goose skin along both sides of the incision.

Thus, it may be said that the results obtained by anæsthetic barriers are substantiated by the method of incision; the fact that there is no material difference in the results obtained by the two methods seems to show that the anæsthetic barriers are complete. Both series of observations definitely suggest that the nervous paths followed by the stimulus in the faradic goose skin reaction are confined to the true skin. This supposition is confirmed by the observations upon cat's skin now to be described.

Some observations upon cat's skin.

Faradic stimulation of the cat's skin[1] yields local goose skin, accompanied by obvious erection of the hairs, over those regions that react to stimulation of the corresponding pilomotor nerves; goose skin is best seen in the shaved skin, upon which we have chiefly worked. The reaction is shown by a patch of skin extending between the eye and the ear, by the skin over the crown of the scalp and by that of the back and tail. The reaction weakens as it is traced from the mid-line of the back towards the ventral surface of the body, being lost in medium sized cats usually about 10 cm. from the mid-line of the back or about half-way to the ventral mid-line. The skin of the limbs does not show it. The patch of skin involved by a single strong stimulation extends over a radial distance of about 1 to 1½ cm. It is much smaller than that seen on human skin, but is of about the same size as that seen in the dog. It is a compact area, in which apparently every hair is raised. Projecting tongues of any appreciable length, or outlying islets have not been identified. As in man, anæsthetisation by injecting the skin quite locally with novocain abolishes the reaction.

It is easy to demonstrate in this animal that the impulse is conveyed

[1] All observations upon these animals have been conducted under full urethane and ether narcosis.

in the skin itself, for the full reaction occurs in pieces of skin freshly excised and laid on the table. That is so if the skin is excised with or without the underlying layer of subcutaneous muscle. The reaction is also present if the excised skin is cleared of the layer of connective tissue intervening between true skin and muscle (see Fig. 7 *i*); it is also present if the inner layers of skin are pared away with a razor. In a series of observations in which the skin so prepared was subsequently examined microscopically, we found that the reaction persisted when a half or even two-thirds of the deeper layers of the true skin was removed, in the last case leaving only the layer of skin which contains the insertions of the erector muscles into the hair roots (Fig. 7 levels *h* and *g*). When the skin is pared until this layer alone remains, the reaction is weakened in force, though it is still extensive.

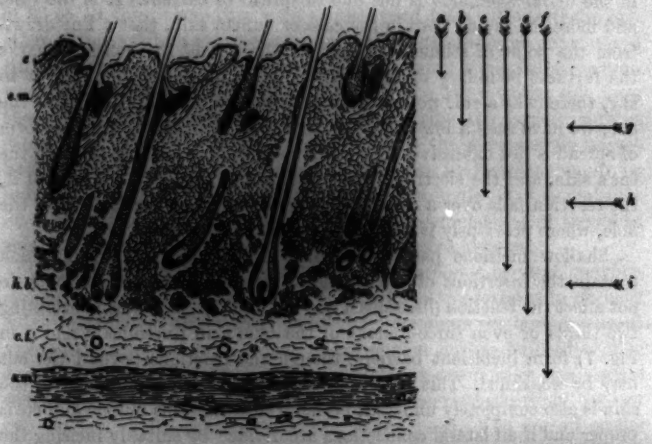

Fig. 7. (×28.) A semidiagrammatic illustration of a vertical section of cat's skin, taken from the back near the middle line. *e* = epidermis; *e.m.* = level of erectors of the hairs; *h.b.* = level of hair bulbs; *c.t.* = connective tissue aponeurosis between true skin and subcutaneous muscle (*a.m.*).

The vertical arrows *a* to *f* represent the depths to which various vertical incisions were carried in different observations. The horizontal arrows *g*, *h*, *i*, illustrate the levels at which the skin was split parallel to its surface in other observations described in the text.

If the skin is cut at right angles to its surface, incisions completely penetrating the true skin always form a complete barrier to spread.

Repeated observations, controlled by subsequent histological examination of the incisions, have shown us that this is so whether the connective tissue layer between true skin and subcutaneous muscle (incisions *e* and *f* in Fig. 7) is divided or not.

If a strip of skin 1½ or 2 mm. wide and 2 cm. long is quickly excised, stimulation of its centre usually yields goose skin throughout the whole length of the strip.

As in the case of human skin, when an incision penetrates the full thickness of the true skin and the edges of the incision are held in full contact with each other, stimulation on one side of the incision yields no reaction on the opposite side. The barrier is effective even if the stimulating electrodes are brought within 1 mm. of the cut. This observation, like those cited earlier, shows that the spread of the reaction in the undamaged skin is not due to spread of current. It is the rule, and more strictly so than in the case of human skin, that a line drawn from the point of stimulation through the end of the incision, marks the farthest boundary of the reaction beyond the incision; that is to say, there is as a rule no spread of the reaction around the ends of the cut; we have seen a few distinct exceptions however, though this form of spread is not extensive. If a small circular hole is made through the cat's skin, and the electrodes are placed immediately to one side of it, the reaction fails over a considerable area of skin on the far side of the hole, where previously it occurred to similar stimulation.

Shallow incisions passing well into or through the layers which contain the insertions of the pilomotor muscles into the hair roots do not affect the reaction (incisions *a* and *b* in Fig. 7). Incisions penetrating two-thirds or even three-fourths of the true skin (incisions *c* and *d* in Fig. 7) form inefficient barriers, though the reactions beyond the cut may be weakened. This statement holds if, in such a preparation, the skin is also completely undercut between subcutaneous muscle and cutis proper and if all lateral connections are divided by suitably incising the skin, thus leaving the stimulated and responding areas in connection with each other through a thin connecting bridge formed by the deeper layers of the true skin only. This experiment is not always successful, however, presumably because the bridge is rendered bloodless and is otherwise damaged by the manipulations; the reaction crosses the bridge only when the preparation is quickly made and is quite fresh.

When considered together, these observations, in which incisions are made vertical to, or parallel with, the skin surface, conclusively show that the reaction of the erector muscles to a faradic current spreads

through a system of nerves lying in and confined to the skin; they further show that this system of nerves is not confined to a particular layer of the skin, but that it is present both in its superficial and deep strata.

Sympathetic system involved. The nerves involved, as might be supposed from our knowledge of pilomotor innervation, belong to the sympathetic nervous system.

Under full ether anæsthesia we have removed the left superior cervical ganglion from three cats. The ganglion and its cephalic branches were defined, the latter cut through separately, and the whole ganglion removed. The wound was sewn up and the animal allowed to recover. All these cats presented the usual ocular manifestations, namely, contracted pupil and paralysis of the third eyelid after operation; and several developed pallor of the left nostril. After survival for periods of 20 to 40 days, these cats were anæsthetised fully with urethane and ether, and the right cervical sympathetic nerve was dissected out, cut and stimulated. The extent of the facial area showing a pilomotor reaction was noted. This and the corresponding area on the opposite side were then shaved, and both were tested by applying the faradic current directly to them. On the left side, to which the sympathetic nerves had been allowed to degenerate, no reaction to faradism could be induced in any of these cats. In all three animals[1] it was perfectly distinct and of its usual extent in repeated stimulations of the right side, to which the sympathetic nerve had been freshly cut.

In three other animals we removed the superior cervical ganglion similarly, and tested the faradic reaction upon the facial skin on both sides immediately afterwards. In all three instances the faradic current yielded distinct and equal areas of goose skin on the two sides. The spread of goose skin from a point stimulated is due neither to a central reflex, nor to a reflex involving the superior cervical ganglion, the last cell station of the pilomotor nerves to the head. The spread occurs provided the peripheral sympathetic nerve fibres are intact, as is here shown and as has been shown by the appearance of the reaction in isolated pieces of skin[2].

To sum up, we may now say that the local goose skin reaction to

[1] In a fourth instance we failed to obtain a distinct pilomotor effect by stimulating the peripheral end of the divided sympathetic nerve or by stimulating the skin faradically on either side.

[2] The last observations, made subsequently, really render the immediate effects of cervical sympathectomy redundant.

faradism is due to spread of impulses in sympathetic nerve fibres that are confined to the skin.

Further observations upon human skin.

Wherever the electrodes are put down on the skin of arm or leg a compact patch of surrounding goose skin is obtained. It is thus apparent that the impulse can move from any single point radially in all directions, and that the direction of movement may be reversed in a short strip of skin by altering the position of the electrodes in relation to such a strip. The same hair may be caused to erect itself by stimulating any one of a series of points arranged circumferentially around it. From these observations it would seem clear that an intricate system of fibres is involved, and that this offers abundant channels of conduction to and fro, the erector muscles of the hairs being presumably innervated by offshoots from this system. The alternative view, that each erector muscle is supplied by distinct nerve fibres from a number of sources sufficiently large to cover the phenomena witnessed is inacceptable. The idea of a network or plexus is also supported by the behaviour of the cat's skin to vertical and horizontal incisions; for these indicate that there are optional channels in the superficial and deep layers of the skin.

If the idea of a sympathetic network is accepted, it can hardly be supposed that this network forms a uniform and continuous sheet in the skin; for, if a small cutaneous nerve is cut, an area of skin subsequently presents the signs of nerve degeneration, since the faradic reaction can no longer be obtained from it; and the area of anæsthesia and of lost faradic reaction correspond closely. It is to be presumed therefore that each separate cutaneous nerve twig, carrying pilomotor fibres, distributes these fibres into its own terminal system. We have no knowledge of the extent of such individual systems, or of the amount by which adjacent systems overlap. We do not know definitely whether functional union is formed at various points between them or not[1].

[1] It seemed that the boundaries of such separate systems might possibly be indicated by stimulating a number of points at short distances from each other in one line and observing the full limits of goose skin obtained from each. If boundaries exist, then sooner or later as the electrodes are moved they should yield goose skin to the margin of one system and, if the next shift of the electrodes does not pass into a region of overlap, the same boundary of goose skin should be maintained. Observation seems to support the idea that such boundaries may actually be present. Successive stimulation of the skin at about 6 mm. intervals in one line, causes a corresponding though irregular movement of the boundary; at certain shifts of the electrodes, however, the boundary remains unaltered, and at the next step, the boundary movement tends to be unusually great.

Projections and outlying islets. As stated earlier, these projections in the form of tongues or islets of goose skin may extend as far away as 10 cm. from the point stimulated. Their direction when definite is usually more or less radial to the point of stimulation; and they usually occur in the length of the limb, though exceptionally they may lie across the limb. Their distribution and behaviour are often puzzling. The first possibilities considered were that long tongues and elongated islets lie in the line of a subcutaneous nerve twig or of a parent strand of sympathetic fibres, the latter in the skin throughout its course, and that direct stimulation of such a strand is responsible for the linear arrangement and long extension of the corresponding goose skin. On further investigation, however, this idea becomes inacceptable. Some little search is usually required to find a point of stimulation that yields not only a compact area of goose skin, but also an outlying islet separated from it by one or more centimetres of unreacting skin. Having located such a point, the islet of goose skin in our experience can be caused to appear from several points of skin in line with it and each other; this observation is consistent with stimulation of a single main strand of nerve fibres possessing no early outgoing branches or connections, but having a small terminal arborisation. But points of stimulation that will yield a given and discrete islet of goose skin a considerable distance away are not confined to a line in the skin; they may be scattered over an area too extensive to permit the supposition that from each of them one and the same nervous strand is directly stimulated. Thus in Fig. 8A the small black points represent the complete area of goose skin appearing on stimulating point *a*. It consists of a relatively compact but elongated area and of an islet of goose skin in the neighbourhood of *k*. It is to be emphasised that a pattern of this kind is not fortuitous, for it is repeated again and again by restimulating at short or long intervals of time (extending to 24 or more hours). An islet of goose skin at *k* could be made to appear on stimulating any of the points marked by a short straight line (as at *a*), though not on stimulating the surrounding points marked by large black circles (as at *i*). Thus it could be provoked from an area of stimulation having a diameter of at least 3 cm.[1]. In this circumstance we cannot assume stimulation of an isolated strand, but must assume excitation of a system of fibres that is united to a strand.

[1] In such instances as that given the islet is not composed in a wholly unvarying manner. As in this instance, the central papillæ of the islet may be erected wherever the stimulus is laid down, but if the stimulus lies more to the left (at *b*) or to the right (at *c*) a few new papillæ are erected to the left or right margin of the islet, respectively.

In other words we must assume an arborisation at each end of one strand. In harmony with this conclusion is the observation that

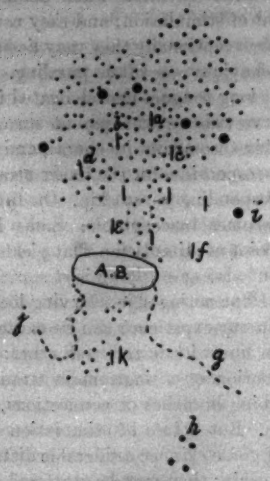

Fig. 8 A.

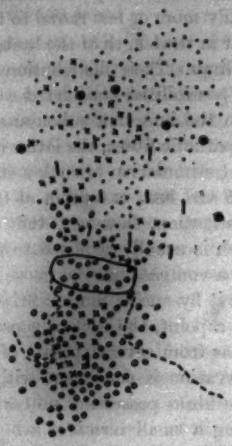

Fig. 8 B.

Fig. 8 A. (×¾.) The small black points represent the hairs erected by stimulating the forearm faradically at point *a*. The islet of goose skin at *h* could be provoked by stimulating any of the 10 points similarly marked by short straight lines between *a* and *f*, but from none of the points marked by black circles (*i* etc.). After placing an anæsthetic barrier in the skin, shown in outline at *A.B.*, the islet of goose skin at *h* could not be produced by stimulating any one of the 10 points referred to above. The lower margin of the goose skin produced in these circumstances by stimulating points *d* and *e* is indicated by line *j*; that produced by stimulating point *f* is represented by line *g* and by four hairs erected as a small islet at *h*.

Fig. 8 B. (×¾.) This diagram shows the same area of goose skin. The black points represent the hairs erected by stimulating at *a*, the plain circles those erected by stimulating at *k*, and the small crosses those erected by stimulating at either *a* or *k*. After putting down the barrier *A.B.* no hairs could be erected above it on stimulating point *k*.

stimulation of point *k* yields, not only a surrounding compact goose skin, but also erects scattered papillæ throughout the area from which the islet *k* can be excited to appear (Fig. 8 B). This is not an isolated instance, but an example of several similar ones observed. In fact it appears to be a general rule that, if an area of goose skin extends by

tongues or islets in a more or less linear fashion, goose skin in greater or lesser degree can be raised along this long line by stimulation at either end of it.

A characteristic of the islet of goose skin is that it is cut off from the central compact area surrounding the point of stimulation by skin in which there is no trace of an erected hair; it is to be observed also that the erection of each hair in the islet may be full. Thus a full stimulus is often conveyed to the responding muscles of the central area and to those in the islet, but none passes to those in the intervening area of skin. A nervous strand, having no connections or no open outgoing connections in its central parts, but having aborisations at its two ends, alone seems to meet this case. Here the idea of a reversible axon reflex must naturally claim attention. There are two possibilities.

In the first place it is possible that a sympathetic nerve fibre passing to the skin divides into two parts, that each of these after a considerable course breaks into an arborisation in the skin, and that stimulation of either set of terminations causes an impulse to travel back to the nerve fibre's division and so through to the other termination. If this view necessitates the supposition that the main axon reflex paths lie deep to the skin, then it can usually be shown to be erroneous. A small barrier of anæsthetic skin formed by intradermal injection of 1 p.c. novocain, laid down across the line joining the islet with the centre of the area from which the islet of goose skin can be provoked, generally prevents the islet from again exhibiting goose skin. When the barrier is put down successfully, its interference is immediate and occurs before the anæsthetic has had time to diffuse into the depth of the skin. The position of a successful barrier is shown in outline in Fig. 8A. It prevented the spread of goose skin beyond the barrier on stimulating *a*. It also prevented the reappearance of the islet *k*, on stimulating the remaining spots individually. This effect was the more remarkable in the case of spot *f*, since a straight line drawn from it to the islet did not pass through the barrier; such a phenomenon has been observed on more than one occasion. Thus we must accept the view that the long path by which the islet is supplied is a nerve path lying in the skin itself, and that islet and central area are not joined by subcutaneous channels; at all events, this must be accepted as usual. On one occasion we have failed to prevent the reappearance of the islet by putting down a barrier of much greater extent than that shown in Fig. 8A. It is of course conceivable that this barrier failed to cross the

skin in which the main nervous strand lay, this path being unusually devious.

The second possibility is that these impulses are carried throughout their whole course in sympathetic networks in the skin, and that in these networks there are paths along which impulses pass with unusual facility. To distinguish between this view and the first we have no substantial evidence.

Comment.

In considering the reaction here investigated it is not to be forgotten that the form of stimulation is artificial; consequently it is to be conceded that the reaction may be of artificial type and without functional significance. Even so it is of interest from several points of view. It seems to be quite necessary, in explaining the phenomena observed, to postulate the conduction of effective impulses along single nervous tracts in both directions; for the terminal systems are certainly capable of conducting effectively in to and fro directions, in fact in all directions. Though the present instance cannot be regarded as completely parallel to sympathetic axon reflexes such as have been described previously by Langley and Anderson(6) and Langley(7), yet it consists of an effective local reflex utilising in part an antidromic channel. The axon reflexes first referred to were in part or wholly preganglionic; that described in the present communication is, so far as we are aware, the only known instance of a purely postganglionic sympathetic reflex.

A second point of interest concerns the intensity with which the hairs are erected in different parts of the area reacting, and the spread of the reaction when the stimulus is strengthened, continued, or repeated. An effective impulse entering a somatic nerve seemingly passes throughout it without decrease of strength. If impulses entering a sympathetic fibre in the skin were similarly conducted, they should pass throughout the whole terminal system to which this fibre belongs and give rise to uniform goose skin over the corresponding area. This is not the case; the impulses decrease in efficiency as they travel. This is shown in two ways; firstly, relatively weak stimulation produces a smaller area of goose skin than does stronger or repeated stimulation; secondly it is the rule for the intensity of goose skin to diminish definitely, though usually somewhat abruptly, in the marginal parts of the reaction. It is to be remarked that we have to deal, not with single impulses, but with repeated ones (induced by faradism) and that these travel through fibres which in our terminal system will often divide and may

possibly reunite. There is the possibility that an impulse, passing through a division, loses strength; there is the possibility that separate impulses approaching by two channels to a point of reunion interfere with each other. Thus the weakness of the marginal goose skin may be due to the musculature here receiving weaker impulses than those received by skin placed more centrally. The artificiality of the faradic current here calls for special notice. We call attention to the fact, however, that a similar phenomenon was reported by Trotter and Davies(2), in the following circumstances. A cutaneous nerve being cut, goose skin was induced through a spinal cord reflex by stimulating skin far removed from the anæsthetic area; the anæsthetic area became surrounded, but was not invaded by, this goose skin; marginally the goose skin was weak. Thus loss of efficiency in travelling is not peculiar to impulses awakened by faradism, but is displayed by natural impulses passing out from the spinal cord. In the faradic reaction there is, however, no uniform relation between the efficiency of the impulses at a given point and the distance of this point from that of stimulation; thus it is not unusual to see full goose skin in an outlying islet of skin, these hairs being more fully erected than those in the marginal parts of the main reaction. Possibly this strong erection at a distant point is due to the confinement of the impulses to unbranching fibres.

These comments are necessarily speculative and our main purpose in making them is to draw attention to the curious manner in which impulses spread in the sympathetic plexuses or networks of the skin; for it is quite possible that information derived from the skin may be relevant to the spread of impulses in nervous plexuses or networks found in other situations, for example in the intestine. The spread of effective impulses in the nerves of the human skin is perfectly displayed by the hairs, and can be studied in a tissue while that tissue remains under unusually natural experimental conditions.

For the moment the chief significance of our observations is practical. The faradic test places at our disposal a simple means of ascertaining in man whether, in instances of nerve lesions, the pilomotor nerves to given areas of skin have degenerated or not. In this respect the test has a value parallel to that of the diffuse vascular flare that follows faradic and other forms of stimulation of the skin; here a sensory nerve axon reflex is involved, the flare failing to appear only when the sensory nerves have degenerated(3).

SUMMARY.

1. If the human skin is stimulated by means of a strong faradic current, goose skin appears for a greater or lesser distance around the electrodes.

2. This goose skin is due to the spread of nerve impulses along a plexus or network of sympathetic fibres which is confined to the skin.

3. The absence of a local reaction of the pilomotor mechanism to faradism should serve to identify areas of skin to which the sympathetic nerve supply has degenerated.

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STUDIES ON THE CIRCULATION RATE IN MAN.

I. Critical examination of ethyl iodide method.

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In 1925 Henderson and Haggard(1) published their description of the ethyl iodide method for the determination of the circulation rate in man. The method has since been investigated by several groups of workers, among whom may be mentioned Cullis, Rendell and Dahl(2), Rosen and White(3), Mobitz(4), Starr and Gamble(5), Davies and Gilchrist(6), Moore, Hamilton and Kinsman(7), Ringer(8) and H. Barcroft(9).

As is well known, the subject breathes an ethyl iodide mixture from a spirometer. Samples of inspired, expired and alveolar air are collected and analysed by the iodine pentoxide method. The pulmonary ventilation is determined. It is assumed by Henderson and Haggard that the partition coefficient of ethyl iodide between alveolar air and arterial blood is 2, and that none (or only a negligible amount) of the ethyl iodide returns to the lungs in the mixed venous blood. The circulation rate can then be calculated from these data.

In the present paper, the authors, who have worked for a considerable time with the ethyl iodide method, propose to describe:

I. Certain modifications which they can recommend in the method of sampling continuously alveolar air.

II. Experiments defining the conditions for storage of uniform mixtures of air and ethyl iodide.

III. Experiments on the estimation of the concentration of such mixtures.

IV. Experiments on the partition coefficient of ethyl iodide, its presence in the venous blood and its destruction in the tissues:

and then to discuss the validity of the method.

(I) Collection of alveolar air.

It is necessary to collect 250 to 300 c.c. of alveolar air for analysis. Henderson and Haggard employed the Müller valve technique, by

means of which, during inspiration, 5–10 c.c. of the air from the end of the previous expiration is sucked over into a 250 c.c. sampling bottle containing air. In about 20 minutes it is assumed that the air in the bottle has been replaced completely by alveolar air. Cullis, Rendell and Dahl(2) have reported favourably on this procedure, but most workers, *e.g.* Ringer(8), Moore *et al.*(7) and Rosen and White(3), have found it necessary to modify the original technique. We have devised an electrical method of sampling, which is simple to operate and has given us reliable results. The arrangement of the apparatus is illustrated in the figures (Figs. 1 and 2). A tube leads from the space

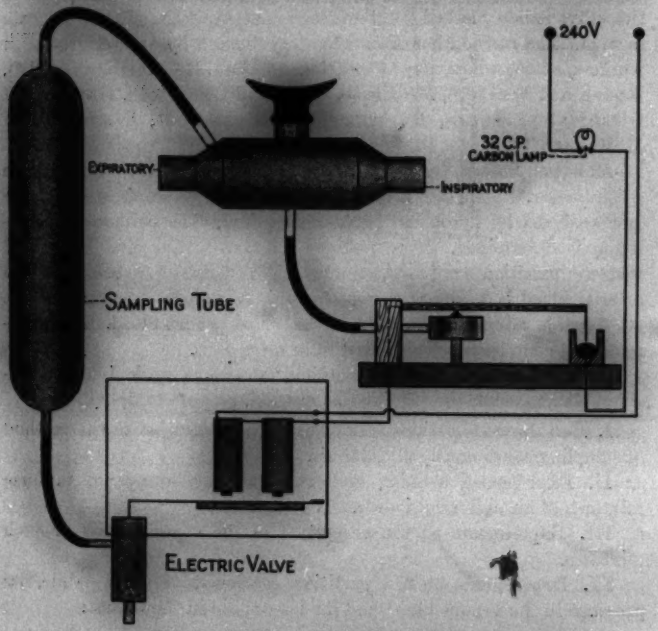

Fig. 1. Arrangement of apparatus for continuous sampling of the alveolar air. *I* = Inspiratory valve. *E* = Expiratory valve. Description in text.

between the valves of the mouthpiece to a small Marey's tambour. The lever which rests on the tambour dips into a mercury cup, the

height of which can be suitably adjusted. The negative pressure of inspiration sucks on the tambour and thus closes the circuit of a large electromagnet, to which is attached the piston of the valve. The valve is made of brass and the rod is of stainless steel. The details of its construction are shown in Fig. 2. There is a tube placed just beyond

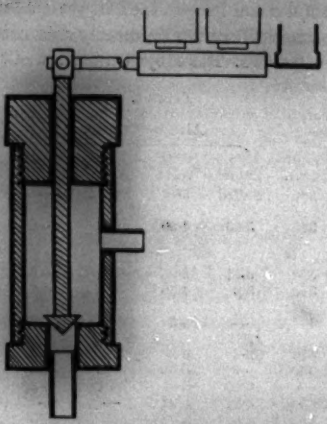

Fig. 2. Detailed structure of valve.

the expiratory valve, which leads to a sampling bottle filled with fluid. During each inspiration the valve piston is raised and a small amount of fluid is permitted to escape. As a result, the air which is present just beyond the expiratory valve, and which thus represents the last of the previous expiration, is sucked over into the bottle. The amount of air collected with each breath can be regulated by a screw clip placed on the rubber tube leading away from the valve. In subjects who salivate excessively, we find that the accumulated saliva interferes with the proper working of the tambour. It is advisable to interpose between the valve and the tambour a small glass "saliva trap," which also serves to prevent the full force of inspiration acting on the tambour. If the lighting circuit is employed, a small carbon lamp must be introduced as a resistance. By means of this valve, two 300 c.c. samples can be collected simultaneously in about 5 minutes.

A series of experiments were carried out to compare the CO_2 content of samples collected by this method and of alveolar air collected at the end of a maximal expiration, using the Haldane-Priestley sampling

tube or the method of Dodds(10). In this latter method the subject is instructed to whistle. As the note begins to fade, the nozzle of a 10 c.c. syringe is introduced between the lips and rapidly filled with air from the mouth. This represents alveolar air. We found that samples collected in this way agree with those obtained by the Haldane-Priestley technique; and samples can be obtained in this manner from subjects in which the other method proves repeatedly unsuccessful. Illustrative results are shown in Table I. The subject was allowed to breathe through

TABLE I.

	Pulmonary ventilation		Alveolar CO_2 p.c.				
Subject	Rate per minute	Depth in c.c.	Before	With valve	After	Difference p.c.	Remarks
T. J.	31	290	Failed	5·14	Failed	—	Untrained. Over-breathing
W. N. H.	22	340	5·86	4·89	5·33	−8	Untrained. Still over-breathing after 15 minutes with valves
M. K.	19	350	6·11	5·95	6·21	−4	—
B. C. D.	21	360	5·8	4·55	4·5 4·2	+5	Over-breathing into Douglas bag
M. K.	16	400	—	5·6	6·3	−12	Breathe into Douglas bag
M. K.	15	470	6·8*	6·0	6·4	−6	After lunch
M. K.	13	630	—	6·36	6·03	+5	—
M. K.	—	—	6·15	6·07	5·91	+2	Cotton wool resistance
S. W.	10	600–800	5·94	5·94	—	—	Cotton wool resistance on inspiratory side; spirometer on expired side
S. W.	8	620	6·35†	6·34	—	—	—
S. W.	9	650	6·07‡	5·92‡	5·78	+2	—

* Mean of 3. † Mean of 4. ‡ Mean of 2.

valves for some time before the continuous sample of alveolar air was collected. The columns headed "Before" and "After" are samples of air collected at the end of maximal expiration just before and immediately after breathing through the valves. It is seen from the table that if the tidal air is 600 c.c. or over, very close agreement is obtained between the two methods. This confirms the finding of Haldane(11), that to obtain a sample of alveolar air, the depth of expiration must be 600–800 c.c. Most untrained subjects, however, breathe through valves in much more shallow manner. We, therefore, give the subject instructions to breathe voluntarily at a slow rate, *i.e.* 10 to 12 times per minute. As pointed out by Haldane(12), if there is no resistance to breathing, the compensation to diminished frequency by increased depth is almost perfect. Adequately deep breathing is thus obtained, and very soon the subject adopts this slow rhythm unconsciously and without effort.

Reference must be made to a recent communication by H. Barcroft(9) in which he has drawn attention to the older work of Krogh and Lindhard(10). These authors claim that there are wide variations in the composition of the alveolar air during each respiratory cycle, and that both the method of continuous sampling and that of Haldane and Priestley yield results which do not represent the average composition of the alveolar air to which the blood is exposed in its passage through the lungs. According to Barcroft the alveolar ethyl iodide tensions obtained by the methods mentioned are lower than the true values, and consequently the circulation rate which is calculated from the use of such data may be 25 p.c. too high. We have not yet had the opportunity to repeat Krogh and Lindhard's observations upon which the argument of Barcroft depends.

II. Storage of ethyl iodide.

Henderson and Haggard advise that the interior of the spirometer in which ethyl iodide is volatilised should be coated with red lead, because rapid destruction occurs if ethyl iodide comes into contact with copper. We have not found red lead very satisfactory. A considerable fall in the concentration of ethyl iodide takes place during one or two hours. Further, as a result of the interaction of the ethyl iodide possibly with the oily basis used in the manufacture of the red lead paint, a compound is formed with a very disagreeable taste and odour, so that after each experiment the interior of the spirometer must be washed out several times with fresh air before it is fit to be used again. We have found that pure nitro-cellulose syrup sold under the name of luc, is more satisfactory. It contains no metallic pigment, and ethyl iodide undergoes little destruction even after prolonged contact. The following data illustrate this point. The concentration of ethyl iodide is expressed throughout this paper in terms of the number of c.c. of $N/200$ sodium thiosulphate employed to combine with the iodine liberated when the ethyl iodide sample was drawn through a heated iodine pentoxide tube; 300 c.c. samples were usually employed.

11 a.m.	Simultaneous samples	4·1, 4·15, 4·3, 4·25	Av. 4·2
12 noon.	„ „	4·0, 4·0	„ 4·0
3 p.m.	„ „	3·8, 3·95, 4·05, 3·9, 4·05	„ 3·95

Ethyl iodide may be left in the spirometer for several days, and the concentration only falls by 20–30 p.c.

Spirometer concentration 6·9; 3 days later 4·75
„ „ 5·0; „ 4·23

After painting the spirometer with luc, it is advisable to have it exposed to the open air for a day or two to drive off traces of the volatile solvent, which is probably amyl acetate.

It is difficult to produce uniform mixing in the spirometer even when an efficient fan is employed for over half an hour. Ethyl iodide is heavy and tends to sink, so that the air coming out of the spirometer first has a higher concentration than that obtained later.

Spirometer reading	2.	Ethyl iodide concentration		6·7, 6·6
„ „	20.	„	„	6·35
„ „	40.	„	„	6·25, 6·25, 6·2, 6·25

After about 100 litres of air have been expelled from the spirometer, it is noted that the readings, though lower, are very constant.

To overcome the difficulty of non-uniform mixing, we employ the electric valve described above to collect 5–10 c.c. sample of the inspired air during each inspiration. The "inspired air" sample consequently represents accurately the air breathed in during the experimental period. We also sample the expired air in the glass mixing chamber in the same way. When determining the circulation rate, we use the valve to collect simultaneously duplicate samples of the inspired, expired and alveolar air.

Ethyl iodide is fairly stable in the presence of water vapour and when in contact with the surface of water, for, as we have seen above, it undergoes very slow disintegration when stored under such conditions in a spirometer. An ethyl iodide mixture can be collected over water and bubbles rapidly through water without appreciable loss. It undergoes very rapid destruction in a Douglas bag.

Example:	A	B
Concentration in spirometer	5·0	5·65
„ „ Douglas bag after 5 minutes	3·25	3·9
„ „ „ „ 20 „	—	2·15

III. Estimation of ethyl iodide.

We have employed the iodine pentoxide method advised by Henderson and Haggard, and use the electrically heated bath manufactured by B. D. H.

(1) *Temperature.* We find the optimum temperature for the reaction is 200–220° C.

(2) *Rate of flow.* We have found in conformity with other workers that the rate of air flow through the tubes of 1 litre per minute (as originally advised) gives unreliable results. Satisfactory estimations can be obtained if an initial slow flow of about 100 c.c. per minute is used

for 3 or 4 minutes. Iodine is deposited on the outlet tube, but is volatilised on running air rapidly through for another 6 minutes. With an initial rapid rate of flow, ethyl iodide can be driven through two iodine pentoxide tubes in succession without undergoing complete destruction.

(3) *Concentration of ethyl iodide.* With concentrations of ethyl iodide equivalent to thiosulphate titrations of 8 c.c. or over, the iodine pentoxide method gives unreliable results, even if an initial slow flow is employed; the tube then remains unreliable for some time after, and requires re-seasoning for several hours.

Experiments to illustrate the effect of rate of air current and concentration of ethyl iodide on the estimation of the latter.

Ethyl iodide in air was run through an iodine pentoxide tube A, then through two KI tubes, and then into a second iodine pentoxide tube B. Samples taken simultaneously from spirometer were used for each pair of estimations.

	Tube A	Tube B	Total
1. 10 minutes at 1 litre per minute	5·25	0·20	5·43
2. 4 minutes slow, 6 minutes fast	6·25	0·14	6·39
1. 10 minutes at 700 c.c. per minute	4·65	0·47	5·12
2. 5 minutes slow, 5 minutes at 700 c.c. per minute	6·15	0·40	6·55
	First 5′	Second 5′	
1. 5 minutes fast (single I_4O_5 tube)	10·5	0·2	10·7
2. 4 minutes slow, 6 minutes fast	—	—	15·88 12·88 14·60

(4) *Seasoning.* We have used iodine pentoxide tubes prepared by the methods both of Rosen and White(3) and of Henderson and Haggard(1). Similar results can be obtained from either kind of tube, but seasoning at 250° C. must be thorough and prolonged. The time given by these authors for the duration of seasoning is too short, and in our experience it may take 72 hours or longer. Seasoning should be continued until close agreement is obtained in the titration for two samples removed simultaneously from the spirometer. Re-seasoning must be carried out at intervals. For no apparent reason the tubes may cease to function satisfactorily at times and require prolonged seasoning.

The iodine pentoxide method has been adversely criticised by Starr and Gamble(5), who have introduced a silver nitrate method for the estimation of ethyl iodide. It must be admitted that the iodine pentoxide method is not easy to employ, but if the precautions enumerated are attended to, simultaneous samples should show agreement within 1 or

2 p.c., or even less at times. With prolonged use, the tubes may give lower absolute readings than previously.

IV. Partition coefficient of ethyl iodide, its presence in the venous blood and destruction in the tissues.

A. *Experiments on animals.* Henderson and Haggard(1) claim that the partition coefficient of ethyl iodide between alveolar air and blood is approximately 2, and that ethyl iodide is destroyed in its passage through the tissues, so that the venous blood contains less than 10 p.c. of the concentration present in the arterial blood. They determined the partition coefficient of ethyl iodide *in vitro* for water and blood, and *in vivo* in experiments on dogs. Starr and Gamble(5) in experiments *in vitro* found the partition coefficient between air and water was 2·7; between air and blood *in vitro* the coefficient was found to be 7·6.

We have endeavoured to determine the partition coefficient *in vivo* in experiments on cats and goats.

In the experiments on cats, the animals were anæsthetised by intraperitoneal injection of 10–15 c.c. of a solution of chloralose saturated at 40° C. The animal breathed in from a spirometer containing varying concentrations of ethyl iodide, ranging from 1·1–7·5 c.c. to 250 litres. The concentration of ethyl iodide used in the determination of the clinical circulation rate is about 0·75 c.c. to 250 litres. It is essential to use much higher concentrations in the animal experiments in order to get an appreciable amount of iodine from the samples of blood. 0·5 p.c. CO_2 was added to the inspired air to increase the pulmonary ventilation. The animal breathed through Müller valves with a very small dead space. Samples of inspired air, expired air, and arterial blood from the carotid artery were analysed for their ethyl iodide content. The expired air was collected in a long glass tube of about 40 c.c. capacity, the outer end of which just dipped under the surface of water in a basin. No attempt was made to collect alveolar air, because the dead space of the smallest rubber valves we had was very large in relation to the tidal air of the cat. The minute ventilation under the influence of the stimulating action of the CO_2 amounted to 360–750 c.c. per minute in some experiments. With this degree of hyperpnœa the expired air approaches fairly closely in ethyl iodide concentration to that of the alveolar air. The expired air bubbled through the water in the Müller valve before being collected in the expiratory sampling tube, but some experiments we made showed that no loss of ethyl iodide resulted from this procedure.

Example: Ethyl iodide mixture from spirometer was driven through dry sampling tubes, then through Müller valve into another series of sampling tubes.

	A	B
(I) Dry bottles	3·75, 3·9	4·95, 5·05
(II) After bubbling through water	3·85	5·35, 5·25

The high figure obtained in II B is probably due to the fact that a certain small amount of water containing ethyl iodide in solution was bubbled over into the sampling tube. As the partition coefficient in water is over 2, the ethyl iodide content of the wet tubes may thus tend to be a little greater than that of the dry tubes.

Analysis of the blood was carried out using the apparatus figured in Henderson and Haggard's paper. A stream of air is bubbled through the blood, and the ethyl iodide passes out of solution and is carried through an iodine pentoxide tube in the usual way. Careful blank determinations were made previously. The apparatus can deal with 5 c.c. of blood quite conveniently, but with quantities of 5–10 c.c. much care has to be exercised because a great deal of frothing takes place, and it is essential that none of the blood shall pass over into the iodine pentoxide tube. We find that a run of 10 minutes, and sometimes of 15 minutes, is necessary to wash out all the ethyl iodide from the blood. Samples of venous blood were taken during the course of the experiment from the saphenous vein, or at the end of the experiment from the inferior vena cava. Samples of arterial blood were also taken after the animal had breathed pure air for 5–15 minutes. The number of c.c. of $N/200$ thiosulphate used in titrating the iodine liberated from the samples of blood examined are given. If the titration is under 0·5 c.c. it is obvious that the margin of error becomes considerable, and less reliance can be placed on the results obtained. The ratio between the ethyl iodide concentration in the arterial blood and in the expired air was calculated. As the ethyl iodide concentration in the alveolar air is lower than in the expired air, the true partition coefficient must be higher than the figures given. The results obtained are summarised in Table II.

In Exp. 5, in which a mixture of 1·1 c.c. ethyl iodide in 250 litres air was employed, the thiosulphate titrations were very low and the results were so markedly irregular that little reliance can be placed on them.

It is clear that concentrations of ethyl iodide at least 4 or 5 times above those employed clinically must be used in this work, in order to get an amount of iodine from the blood samples which bears a reasonable relation to the blood blank. Considerable variations were found

TABLE II. Experiments on Cats.

Expt.	Ethyl iodide in 200 l. spirometer air c.c.	Percentage CO_2 in spirometer	Concn. of ethyl iodide in 100 c.c. arterial blood during expt. (in terms of N/200 thiosulphate)	Ratio of ethyl iodide in arterial blood to that in expd. air: Average	Range	No. of determinations	Percentage return in venous blood	Remarks
1	7·5	0·5	32·3–39	3·0	2·7–3·2	2	75	After breathing fresh air for 5 mins. arterial blood still contained 45 p.c. of previous ethyl iodide concentration
2	6·0	0·5	32·5–56	6·1	4·2–7·1	3	70	Arterial blood contained 35 p.c. of previous ethyl iodide concentration after breathing fresh air for 15 minutes
3	5·0	0·5	20·0–41·3	4·3	3·4–5·6	4	75	—
4	5·0	—	10·3–48·0	4·4	3·5–5·4	2	40	—
5	1·1	0·5	3·7– 6·5	—	1·8–5·6	3	—	Thiosulphate titrations very low (0·15–0·75 c.c.). Results very irregular

in the ratio of ethyl iodide in arterial blood to that in expired air. The average values in Exps. 1 to 4 were 3·0, 6·1, 4·3, 4·4; the range being from just under 3·0 to slightly over 7. The actual partition coefficient must be higher than these figures. A large return of ethyl iodide (from 40–70 p.c.) takes place in the venous blood. Ethyl iodide was still present in the arterial blood after the animal had breathed pure air for 5–15 minutes. In some of the experiments the concentration of ethyl iodide in the arterial blood increased steadily during the course of several hours, although the pulmonary ventilation showed no similar change.

In the experiments that were carried out on 5 goats, the animals breathed through valves, and alveolar air was collected at the same time as the arterial blood samples. A "spear-valve" respirator made by Siebe-Gorman was employed. This had a small dead space, and the valves had almost no resistance whatever. Owing to the very long trachea in the goat, the total dead space in our experiments was found to be high—at least 75 c.c. in one animal with a tidal air of about 115 c.c. It is therefore probable that real alveolar air was not collected and that the samples obtained represented a mixture of alveolar and some dead space air. The ethyl iodide concentrations found in the sample are in consequence too high, and the real partition coefficient is greater than the figures obtained.

Example:

Goat. 17 Kg. 2·5 c.c. C_2H_5I 1 to 250 litres air. No CO_2

3.40. Breathing started

3.45. 6 c.c. arterial blood = 0·56 c.c. thiosulphate = 9·3 c.c. per 100 c.c.
Alveolar air = 1·54 " = 2·6 "
Expired air = 5·55 " = 7·9 "

3.55. 6 c.c. arterial blood = 0·58 " = 9·5 "

Partition coefficient $\frac{9{\cdot}4}{2{\cdot}6} = 3{\cdot}6$.

When the inspired air contained 5·0 c.c. C_2H_5I in 250 litres air the arterial blood concentration was 19·4 c.c. thiosulphate per 100 c.c. and the partition coefficient was 3·4. With 10 c.c. ethyl iodide in 250 litres air the arterial blood contained 16·4 and 18·0 c.c. thiosulphate per 100 c.c. and the partition coefficient was 3·0.

It is to be expected that in animal experiments of this character considerable variation in the results should be obtained. The character of the breathing influences the degree and uniformity of ventilation of the alveoli. The *p*H of the blood may perhaps affect the partition coefficient. The anæsthetic may produce changes in the permeability of the alveolar epithelium, and if there is œdema of the lung, interference occurs with gaseous interchange with the blood. Thus in one experiment in a goat when there was much mucus in the trachea and pulmonary œdema, a partition coefficient of 1·4 was obtained. Atropine was administered; then the experiment was repeated 30 minutes and 50 minutes later, and coefficients of 2·8, 2·7 were obtained. The range of results in the goats was 2·0–3·6, the higher results being obtained in the more satisfactory experiments.

These experiments of course do not tell us what the partition coefficient is in man when the ethyl iodide concentrations used clinically are inhaled for a short time.

B. *Return of ethyl iodide in the venous blood in man.* A series of experiments were carried out to determine whether ethyl iodide returns in the venous blood in man. Two methods were employed to test this point.

(1) The subject breathed from the spirometer, and the expired air was collected in a glass mixing chamber of 6 litres capacity. A period of 3–6 minutes was allowed to wash out the air originally present in the chamber, and then samples of alveolar and expired air were collected at 5 minute intervals for periods of 20–30 minutes. If ethyl iodide returns in the venous blood, there should be a definite rise in the concentration of the vapour in the samples collected. Any variation that

occurs in the extent of the pulmonary ventilation must be taken into account. The results obtained are given in Table III.

TABLE III.

Subject	Ethyl iodide per 300 c.c. inspired air (in terms of N/300 thiosulphate) c.c.	Preliminary breathing mins.	Time of collection of samples	Ethyl iodide per 300 c.c. expired air c.c.	Ethyl iodide per 300 c.c. alveolar air c.c.	Pulmonary ventilation per min. litres	Remarks
R. W. P.	4·06	5	Next 5 mins.	1·43	0·90	6·08	—
			" 5 "	1·36	1·01	6·5	—
			" 5 "	1·45	0·99	7·1	—
			" 8 "	1·70	1·07	6·02	—
A.	5·89	6	" 5 "	2·07	1·25	9·3	Definite rise of alveolar and expired concentrations
			" 5 "	2·71	1·66	8·2	
			" 5 "	2·71	1·79	8·8	
			" 5 "	2·83	2·05	8·3	
M. K.	6·50	3	" 5 "	1·65	1·11	5·3	First sample low because taken too soon
			" 5 "	2·64	1·51	4·7	
			" 5 "	2·77	1·46	6·05	
			" 5 "	—	—	6·2	
			" 6 "	2·63	1·83	6·35	
P.	2·44	5	" 5 "	1·09	—	6·9	Pulmonary ventilation diminished but expired air unchanged
			" 5 "	1·01	—	6·05	
			" 5 "	1·12	—	5·95	
			" 5 "	1·09	—	5·65	
			" 10 "	1·13	—	5·63	

In the experiment on A. there was a continuous rise in the ethyl iodide concentrations in the alveolar and expired air, though the pulmonary ventilation diminished during the period of observation. In the case of P., the expired air concentrations were steady though the pulmonary ventilation fell by 20 p.c. The other two experiments are inconclusive. The ethyl iodide concentrations used in the spirometer were those used clinically or less.

(2) The subject breathed for 7 to 10 minutes from the spirometer. A sample of the alveolar air was collected during the last 2 minutes. A series of maximal respirations were taken, and samples of alveolar air were collected at intervals. Illustrative results are given below. Dr Douglas informed one of us that in his experience 12 maximal respirations are sufficient to wash out a foreign gas which is present in the alveoli.

Exp. 1. R. W. P. 2·5 c.c. ethyl iodide to 250 litres in spirometer:

	Alveolar air collected	Ethyl iodide in 300 c.c. c.c.
7 minutes breathing from spirometer	During last 2 minutes	3·3
3 " fresh air (13 deep breaths)	" 4 breaths	2·81
3 " " (12 ")	" "	2·10
2 " " (6 ")	" "	1·60

Duration of after breathing—8 minutes.

Exp. 2. 1·25 c.c. ethyl iodide in 250 litres in spirometer:

	Alveolar air collected	Ethyl iodide in 300 c.c. c.c.
10 minutes breathing from spirometer	During last 2 minutes	4·75
3 „ fresh air (24 deep breaths)	„ 6 breaths	2·20
3 „ „ (24 „)	„ 6 „	1·05
3 „ „ (24 „)	„ 6 „	1·0

Duration of after breathing—9 minutes.

Exp. 3. 0·75 c.c. ethyl iodide to 250 litres in spirometer:

	Alveolar air collected	Ethyl iodide in 300 c.c. c.c.
10 minutes breathing from spirometer	During last 2 minutes	2·19
3 „ fresh air (26 deep breaths)	„ 8 breaths	0·70
3½ „ „ (27 „)	„ 9 „	0·70
3 „ „ (25 „)	„ 7 „	0·65

Duration of after breathing—9½ minutes.

It is clear from these experiments that ethyl iodide is still being given off from the mixed venous blood into the alveolar air after fresh air has been breathed deeply for periods exceeding 9 minutes. If it is assumed that the samples collected during this time are in equilibrium with the arterial blood, then it follows that the latter contains considerable concentrations of ethyl iodide for many minutes after the inhalation of the vapour is concluded. If the ethyl iodide concentration in the arterial blood when breathing spirometer air be taken as 100, then in Exp. 1, when fresh air was breathed, the arterial blood contained 34, 25 and 20 after 3, 6 and 8 minutes respectively. In Exp. 2, it contained 46, 22, and 21, and in Exp. 3, it contained 32, 32 and 29, after 3, 6, and 9 minutes respectively. Similar results were obtained in Exps. 5 and 6, quoted in the succeeding paragraph. It is clear that towards the end of the period of inhalation of the mixture there was a return of at least 30–50 p.c. in the mixed venous blood.

This agrees with the results of the animal experiments discussed earlier. It should be noted that in Exps. 2, 3, 5, 6, low concentrations of ethyl iodide were employed (0·5–1·25 c.c. in 250 litres of air).

C. We carried out some experiments in which the subject breathed an ethyl iodide mixture for 15–25 minutes. He then breathed fresh air, and the expired air was collected for the next 15–20 minutes, and its ethyl iodide content determined. The total amount exhaled during this period is not large, and represents 2–5 p.c. of the ethyl iodide previously absorbed. It seems probable that the ethyl iodide which escapes from the blood into the tissues does undergo destruction there and does not

accumulate to any extent, otherwise a much larger amount would be given off in the expired air when fresh air was breathed after the experimental period. Details of some illustrative experiments follow:

Exp. 4. R. W. P. 0·5 c.c. ethyl iodide in 250 litres; breathed for 17 minutes. Inspired 147·5 litres (300 c.c. = 5·24 c.c., *N*/200 thiosulphate); expired air, 300 c.c. = 2·62 c.c.; ethyl iodide absorbed = 1813 c.c.

Fresh air now breathed 86·5 litres in 13 minutes; expired air 300 c.c. = 0·13 c.c. thiosulphate. Ethyl iodide exhaled = 40 c.c.

Alveolar air after 7 minutes = 0·30 c.c. per 300 c.c., after 12 minutes = 0·20 c.c., after 17 minutes = 0·08 c.c.

Exp. 5. Pearce. 0·75 c.c. ethyl iodide in 250 litres; breathed in 23·5 minutes, 107·5 litres. C_2H_5I absorbed (3·94 per 300 c.c.) = *1408* c.c.

Fresh air, 12 deep respirations; then started collecting alveolar and expired air.

C_2H_5I exhaled first 9 minutes = *22* c.c.
„ „ next 8·5 „ = *10* c.c.

C_2H_5I in alveolar air when breathing from spirometer = 0·92 c.c. per 300 c.c.

After 5 minutes of fresh air = 0·17; after 17 minutes = 0·10.

Exp. 6. Ascroft.

From spirometer, breathed in 22·5 minutes, 136 litres. C_2H_5I in inspired air = 8·59 c.c. per 300 c.c.; in expired air = 3·68 c.c.; absorbed *2226* c.c.

Fresh air, 12 deep respirations; then started collecting alveolar and expired air:

C_2H_5I exhaled in 5 minutes = *58* c.c.
„ „ next 11½ minutes = *30* c.c.

C_2H_5I in alveolar air when breathing from spirometer = 2·54 c.c. per 300 c.c.; after 5 minutes of fresh air = 0·70; after next 11·5 minutes = 0·35.

Discussion.

From the results of the experiments described above, it is clear that the partition coefficient of ethyl iodide between blood and air *in vivo* may vary from over 2 to over 7, and is thus considerably higher than that described by Henderson and Haggard. We cannot say, however, what the partition coefficient is in man after breathing the clinical concentrations of ethyl iodide for a period of 20 minutes.

We have also shown that there is a very considerable return of ethyl iodide in the venous blood when high concentrations are breathed, and a definite return when low concentrations are used. Our results also suggest that the extent to which ethyl iodide returns in the venous blood varies with the subject, and in the same individual under different conditions. Any increase in the circulation rate, general or local, *e.g.* in the vessels of the skin, would probably tend to cause a greater return in the venous blood.

It thus appears that the fundamental propositions on which the ethyl iodide method is founded are invalid.

It is claimed by some workers, however, that the method may be empirically sound and the results obtained, though not representing the absolute circulation rate, are comparable. These authors claim, and we confirm this to some extent from our own experience with the method, that if the circulation rate is repeatedly determined in one subject under standard conditions, fairly uniform results are obtained. This seems to us to prove very little, because we admit that probably any individual under the same circumstances deals with ethyl iodide in a uniform manner. But the results would not be comparable with those obtained in the same individual under other conditions, nor are they comparable with the results obtained in other subjects who may deal with ethyl iodide in a different manner. We find, too, as other workers have done, that when the circulation rate is determined by the ethyl iodide method and by the Fick principle, results of the same order of magnitude are obtained (though not without marked exceptions). As pointed out by Starr and Gamble, this agreement may be due to the return in the venous blood nullifying the effect of the high partition coefficient.

The ethyl iodide method appears to be unsuitable for determinations of the output of the heart during exercise even for comparative purposes. With the rapid circulation rate, the return in the mixed venous blood is even greater than it would be in the same subject at rest.

We do not know in what form ethyl iodide is carried in the blood. In our experiments on the partition coefficient, we observed that when air is bubbled through the blood there is a small liberation of ethyl iodide in the first few minutes, and that a much larger quantity comes off in the next 5 or 10 minutes. Possibly ethyl iodide may exist in the blood in two forms, (*a*) in solution, (*b*) in some more stable state, perhaps forming a loose compound with one of the constituents of the blood.

Summary.

(I) An electrical method is described by means of which continuous sampling of the alveolar air can be carried out. It can also be employed to sample automatically and simultaneously the inspired and expired air as well.

(II) The estimation and properties of ethyl iodide are discussed.

(III) The partition coefficient of ethyl iodide *in vivo* in anæsthetised animals has been shown to be between about 2 and 7.

(IV) Ethyl iodide returns in the venous blood in considerable amounts

both in animal experiments and in the human subject. It undergoes rapid destruction in the tissues.

(V) The validity of the ethyl iodide method for determining the circulation rate is discussed.

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THE REACTION BETWEEN ACETYL CHOLINE AND MUSCLE CELLS. Part II.

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Section I. The destruction of acetyl choline by the frog's heart.

In a previous paper(1) the writer described the relation between the concentration and the action of acetyl choline on the isolated heart and on the rectus abdominis of the frog. Loewi and Navratil(2) have since shown that extracts of frog's tissues and particularly extracts of the frog's heart can destroy acetyl choline fairly rapidly. This suggested a possible source of error in the writers' calculations both regarding the relation between concentration and action, and the amount of drug fixed by the heart, and therefore experiments were made to eliminate these errors.

Experimental methods. The following methods were used:

(1) Immersed strip: a strip of ventricle was immersed in Ringer and the isometric response was recorded.

(2) Moist strip: a moist strip of ventricle was suspended in air and its response recorded isometrically. This method was used chiefly to estimate the amount of acetyl choline present in very small quantities of fluid.

(3) Irrigated strip: a strip of ventricle was arranged as above, but was irrigated by a fine jet of fluid driven by compressed air. Two capillary nozzles were arranged so that irrigation either with a solution of a drug or with Ringer's fluid could be alternated rapidly.

(4) Isometric ventricle: the response of the whole ventricle was recorded isometrically. The arrangement used is shown in Fig. 1.

The advantages of method (4) are that any quantity of irrigation fluid from 10 c.c. to 0·1 c.c. can be used, and can be changed quickly. The whole system outside the heart was filled with boiled saline (0·65 p.c. NaCl). The usual initial tension was 3 cm. of water, and this sufficed to produce a rapid diastolic filling. The ventricle was allowed to contract isotonically except for short periods when records were taken, and thus the heart received an adequate amount of aerated fluid.

In all four methods the heart was driven at a regular rate (about 15 per minute) by break induction shocks. The Ringer's fluid had the

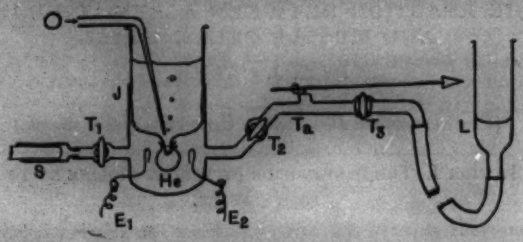

Fig. 1. Apparatus for measuring action of drugs on isometric response of frog's ventricle with varying initial fillings, or varying initial tensions.

He = heart; *O* = oxygen supply; T_1, T_2, T_3 = glass taps; E_1, E_2 = electrodes; *J* = ground glass joint; *Ta* = small rubber membrane tambour (diameter 5 mm.); *S* = all glass syringe, graduated to 0·01 c.c., for varying initial filling; *L* = moveable level for varying initial tension.

following composition: NaCl 0·65 p.c.; $CaCl_2$ 0·012 p.c.; KCl 0·015 p.c.; sodium phosphate at *p*H 7·5 0·05 p.c. A stock concentrated solution of phosphate at the desired *p*H was used.

The relation between concentration and action of acetyl choline. The irrigated strip method eliminated errors due to destruction of the drug, since the heart cells were irrigated continuously with fresh solution. The curve relating action and concentration obtained with this method was identical with that described in my previous paper(1), which was obtained with the immersed strip method. The only difference was that a given concentration of drug produced a much greater effect with irrigation than with immersion. For instance in one heart where the two methods were tested alternately, a 50 p.c. reduction in the force of contraction was produced by 10^{-7} molar with irrigation, whereas 10^{-6} molar was needed to produce the same effect with simple immersion. This difference depends presumably on the fact that with simple immersion the drug only diffuses slowly into the sponge-like tissue of the ventricle and as it is being broken down there continuously, the actual concentration on the cell surfaces is much less than that in the bulk of the fluid.

The measurements made from strips of ventricle are, however, open to the objection that the force of contraction only represents a small fraction of the maximum force the ventricle can exert. Experiments

were therefore made with the whole ventricle contracting isometrically. When the initial filling was adequate, the maximum systolic tension of a fresh heart was usually 70–90 cm. of water.

This method was found to give satisfactory results and the curves obtained relating concentration and action were the same as those described in my previous paper (1). Fig. 2 shows the results of a typical experiment.

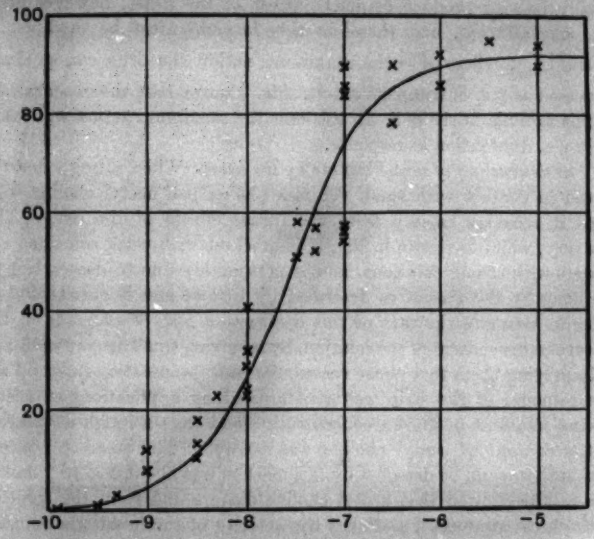

Fig. 2. Action of acetyl choline on the isometric response of the frog's ventricle.

Ordinate (y) = Reduction in force of contraction, expressed as percentage of normal contraction.

Abscissa = Logarithm of (x), the molar concentration of acetyl choline.

The crosses show the observed figures whilst the curve is drawn to the formula $k.x = \frac{y}{92-y}$. ($k = 10^{7.4}$.) In the figure "x" is shown on the logarithmic scale.

The results obtained with the improved methods described agree therefore with the results obtained with the simpler strip method, but the more accurate methods of recording show that in most cases acetyl choline does not produce complete arrest of the heart, for usually there

is a small residual contraction which is not abolished even when very high concentrations of the drug are employed.

In my previous paper the formula adopted for interpreting the relation between concentration and action was $K \cdot x = \frac{y}{100-y}$ where K = constant, x = concentration and y = action produced by the drug expressed as p.c. of the maximum possible action: this last was taken to be complete arrest. The more recent methods show that the drug usually fails to produce complete arrest of the heart, however great the concentration, and therefore this formula must be modified to $K \cdot x = \frac{y}{A-y}$, where A = the maximum action the drug can produce, expressed as p.c. of complete arrest. Fig. 2 shows that the observations fit this formula fairly well. In this case the maximum action was taken as 92 p.c. diminution in response.

The destruction of acetyl choline by the heart. When a frog's heart is placed in contact with small volumes (0·5 c.c.) of acetyl choline solutions it recovers rapidly from the initial effects of the drug. This recovery, which is shown in Fig. 3, is quickest with weak solutions and slowest with strong solutions, and is presumably due to destruction of the drug by the tissues as described by Loewi and Navratil(2). In order to estimate the rate of this destruction tests were made of the acetyl choline content of the solution, by removing small drops (0·005 c.c.) and applying them to a moist ventricular strip preparation (method *A*). The response of the strip was standardised by applications of acetyl choline solutions of known concentration and thus a rough estimation of the content of acetyl choline was possible. The smallest concentration that could be detected by this method was about 5×10^{-7} molar. The results showed that acetyl choline disappeared from the solution as the heart recovered, and that the activity of the heart was roughly proportional to the content of acetyl choline remaining in the fluid.

Straub(3) described a similar type of recovery from muscarine in the hearts of aplysia, torpedo and the frog. He showed firstly that in the case of aplysia the drug passed into the heart and was stored there, and secondly that the heart when it had absorbed the drug became tolerant to further applications.

In the case of acetyl choline I was unable to detect any storage of the drug in the frog's heart. To test this point hearts were exposed to excess of acetyl choline (10 c.c. of 1[illegible] molar) for periods of one to twelve hours. At the end of such periods there was still a considerable concentration of drug remaining in the solution, but when the heart

was rinsed with Ringer's fluid and then ground up with sand, no acetyl choline could be detected in the emulsion.

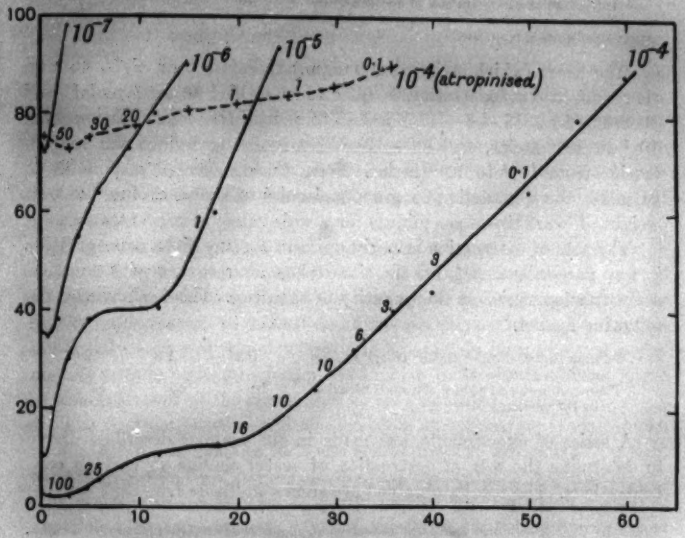

Fig. 3. The destruction of acetyl choline by the frog's ventricle.

Abscissa = Time in minutes.

Ordinate = Isometric contraction expressed as p.c. of normal.

In all cases quantities of 0·5 c.c. were introduced into a ventricle which weighed 100 mgm. (moist weight). The curves show the response of the heart after concentrations of acetyl choline varying from 10^{-7} to 10^{-4} molar, and also the response to 10^{-4} molar acetyl choline after the ventricle had been atropinised.

Drops of fluid were removed at intervals and their content of acetyl choline tested on a ventricular strip. The figures along the curves show the molar concentrations of acetyl choline $\times 10^6$.

The frog's heart acquires a certain amount of tolerance to acetyl choline on prolonged exposure to the drug. This effect, which will be discussed later, is a source of error in the calculation of the rate of destruction of the drug but does not affect the main conclusions that the frog's heart destroys acetyl choline at a fairly rapid rate.

In the experiment shown in Fig. 3, 10^{-7} molar acetyl choline produced a 30 p.c. reduction in the force of contraction of the heart and

the times taken by the heart to recover after varying doses of acetyl choline to a response 70 p.c. of normal, were as follows:

Initial molar concentration of acetyl choline	10^{-6}	10^{-5}	10^{-4}
Time in minutes taken to recover to 70 p.c. of normal response	8	19	52

The heart (which weighed 100 mgm.) therefore took eight minutes to reduce the concentration in 0·5 c.c. from 10^{-6} to 10^{-7} molar, and therefore took $19 - 8 = 11$ minutes to reduce the concentration from 10^{-5} to 10^{-6} molar, and $52 - 19 = 33$ minutes to reduce the concentration from 10^{-4} to 10^{-5} molar. From these figures it is possible to calculate the destruction in gram molecules of acetyl choline per unit weight of heart tissue per minute for a wide range of concentration.

The rate of destruction is fairly uniform for any given concentration in any particular heart, for the time taken for recovery with constant concentration varies as the quantity of the drug. This is shown by the following figures:

Volume in c.c. of 10^{-7} molar acetyl choline solution introduced	0·2	1·0	2·0
Time in minutes to reduce the concentration to 10^{-6} molar	10	45	80

A series of experiments was made in the manner described above to determine the rate of destruction of acetyl choline at varying concentrations. The results obtained are shown in Table I.

TABLE I. Time in minutes taken for reduction of molar concentration of acetyl choline. All figures reduced to common standard of 0·1 c.c. fluid in a ventricle weighing 100 mgm.

Date	10^{-3}–10^{-4}	10^{-4}–10^{-5}	10^{-5}–10^{-6}	10^{-6}–10^{-7}	10^{-7}–10^{-8}
16. xi. 26	—	—	—	5·7	2·8
17. xi. 26	7	6·2	3·3	4·5	2·0
25. xi. 26	—	8	3·5	2·4	1·6
9. xii. 26	6	6·6	2·2	1·6	—

These figures were confirmed by other experiments made upon isolated strips of ventricle to which drops of fluid were added. The rate of recovery of these strips indicated a destruction of acetyl choline at a rate of the same order as that described above. The figures in Table I show that there is a considerable individual variation in the rate of destruction of the drug, but that in all cases the time required to reduce the concentration to one-tenth is three or four times greater with the highest than with the lowest concentrations. This difference is remarkably small considering that the concentration varies ten thousand-fold.

TABLE II.

Range of molar conc. of acetyl choline	10^{-3}–10^{-4}	10^{-4}–10^{-5}	10^{-5}–10^{-6}	10^{-6}–10^{-7}	10^{-7}–10^{-8}
Gram molecules of drug destroyed per minute per mgm. of heart (moist weight)	2×10^{-10} to 10^{-10}	10^{-11} to 3×10^{-12}	4×10^{-12} to 10^{-12}	6×10^{-13} to 10^{-13}	10^{-14} to 2×10^{-14}

The average figures given in Table II show that within the limits of error the log. of the amount of drug destroyed plotted against the log. of the concentration of the drug gives a linear relation, and the relation between the amount destroyed (x) and the concentration (c) can be expressed by the formula $K.c^{1/n} = x$; where $K = 7 \times 10^{-8}$, and $n = 1{\cdot}2$.

The rate of destruction of acetyl choline varied considerably in different hearts, for some hearts destroyed the drug ten times as quickly as others. Variations in the rate of destruction of the drug did not bear any certain relation to variations in the sensitivity of the heart. One abnormal heart was found which was completely insensitive to acetyl choline even in concentrations of 10^{-4} molar, but the rate of destruction in this heart was of the same order as that in normal hearts.

The destruction of acetyl choline after atropinisation is also shown in Fig. 3. In this case the ventricle was exposed to atropine 10^{-5} molar; the atropine was then washed out, leaving the ventricle very insensitive, and the rate of destruction of acetyl choline was tested on a strip of ventricle. The rate of destruction in this case was within the limits of variation of figures obtained with normal hearts.

The fact that destruction of acetyl choline proceeds unaltered both in atropinised hearts and in hearts naturally insensitive to the drug suggests that there is no direct relation between the amount of drug destroyed and the amount of action the drug produces.

Loewi and Navratil(2) showed that emulsions of heart tissue destroyed acetyl choline, and that this action was abolished by heating to 56° C. They also showed that acetyl choline was destroyed by emulsions of liver and gut and to a lesser extent by emulsions of skeletal muscle. I confirmed these results as regards emulsions of the heart, liver, gut and skeletal muscle and also found that frog's serum had as powerful an action in destroying acetyl choline as the frog's heart, for the destruction by 0·001 c.c. serum per minute was of the same order as the destruction by 1 mgm. of moist ventricle per minute. My experiments confirm Loewi and Navratil's conclusion that the destruction of acetyl choline is due to a ferment, and that this ferment is widely

distributed in the frog's tissues, and is not confined to those tissues on which the drug produces a specific action. Many similar ferments that destroy other drugs are known. For example, the frog's heart, liver and serum contain a ferment which destroys atropine (4).

The destruction of acetyl choline by the heart appears to be due to an intracellular ferment, for I found that when 0·2 c.c. of fluid was kept in a heart for two hours and then removed, the fluid had no power to destroy acetyl choline. Moreover, the action is not due to any substance that can be washed out of the heart, for a heart that had been isolated for 24 hours and had its perfusion fluid changed at least one hundred times still retained its full power to destroy the drug.

The amount of acetyl choline reacting with the frog's heart. The writer has calculated (1) the amount of acetyl choline actually reacting with heart cells by comparing the effects of the drug upon a strip of frog's ventricle immersed in a large volume of solution with the effects produced when small quantities of drug are added to the moist ventricular strip. This method showed conclusively that the amount of drug actually reacting with the heart cells must be very small. The fact that the heart cells can destroy acetyl choline fairly rapidly further reduces the possible quantity of drug that can react with the cells.

Table III shows in line 2 the figures calculated in a previous paper (1) for the amount of drug disappearing from solution when the drug acted on heart cells, and in line 3 are shown the quantities which can be attributed to destruction of the drug by the ferment action already described. A comparison of the figures given in Table III shows that by this calculation the destruction of the drug would account for nearly the whole of the drug disappearing at the lowest concentration measured, but that at higher concentrations the amount destroyed is only a small fraction of the quantity that disappears.

TABLE III.

(1) Molar concentration of acetyl choline added	10^{-7}	10^{-6}	10^{-5}	10^{-4}
(2) Gram mols. acetyl choline disappearing per mgm. of dry tissue	5×10^{-14}	6×10^{-13}	$1{\cdot}6 \times 10^{-10}$	$1{\cdot}6 \times 10^{-9}$
(3) Gram mols. of drug destroyed within 1 minute per mgm. of dry tissue	3×10^{-14}	8×10^{-13}	3×10^{-12}	3×10^{-11}

It is of course unjustifiable to assume that the whole of the drug that disappears without being destroyed by the ferment action necessarily takes part in producing the specific action of the drug. A fixation or adsorption of pilocarpine and other drugs by serum and other tissues

on which the drugs exert no specific action has been described (Storm van Leeuwen(5), Beutner(6)).

The figures given in my previous paper for the maximum amount of drug that can possibly react with the tissues to produce the specific action of the drug are therefore too high, and there is a considerable probability that the true figures are very much smaller.

Rate of reaction and wash out of acetyl choline. Experiments with isolated strips upon which a jet of solution was played made it possible to measure approximately the rate of reaction and the rate of wash out of the drug. The following figures were obtained:

Molar concentration acetyl choline	10^{-3}	10^{-4}	10^{-7}
Time of half action	1·4″	2·0″	3·7″
„ full action	4″	6″	9″
„ half wash out	3″	<3″	—

These figures show that the combination between acetyl choline and the tissues occurs very rapidly and that the drug can be removed equally rapidly by washing out.

If the jet of acetyl choline solution be stopped, and the strip is not washed with Ringer, the heart recovers owing to the destruction of the drug. This recovery due to destruction of the drug is a much slower process than washing out, as is shown by the following figures:

Molar concentrations of acetyl choline	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Time for half recovery on washing out	—	<3″	3″	12″	24″
Time for half recovery due to destruction of the drug	10″	12″	25″	80″	117″

The rapidity with which the action of acetyl choline is produced on introduction of the drug, and is removed by washing out, supports the view that the drug acts on the surface of the cells.

Tolerance to acetyl choline. Straub(3) found that when the heart of aplysia was exposed to muscarine the drug was concentrated in the heart cells, and that the heart then became tolerant to the drug. He showed a similar tolerance in the frog's heart and concluded that the action of the drug depended on the difference of concentration without and within the cells. Gasser and Dale(7) found that the rectus abdominis of the frog became insensitive to acetyl choline upon prolonged exposure to the drug.

I observed a partial recovery of the frog's heart after exposure to a constant concentration of acetyl choline. This effect is shown in Fig. 4; in this case the quantity of solution employed was too great for the destruction of the drug by the heart to produce a significant alteration in the concentration. The figure shows that the drug produces its

maximum effect in from 15 to 30 seconds, and that this is followed by a partial recovery which is completed within about 10 minutes. This effect is seen also in Fig. 3, where there is a rapid initial recovery due to tolerance, which is followed by a slower continuous recovery due to destruction of the drug.

Other experiments showed that this tolerance only produced a partial recovery, and that, when a heart was exposed to the drug for periods of some hours, no further recovery occurred after the first ten minutes, provided of course that effects due to destruction of the drug were excluded.

It seems unlikely that this tolerance is due to storage of the drug in the heart, because, as has been previously mentioned, no such storage can be demonstrated, and moreover is very improbable, in view of the power of the heart to destroy the drug.

The full sensitivity of the heart to acetyl choline is recovered rapidly on washing out, and experiments made with rapidly moving drums showed that after washing out for even 30 seconds the full sensitivity of the heart was restored. It appears unlikely that it should be possible to remove the drug from the interior of the cells at this speed.

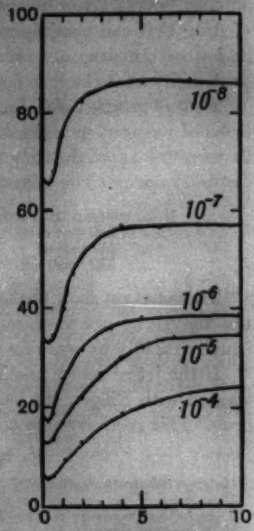

Fig. 4. Response of isometric ventricle after introduction of 10 c.c. acetyl choline solutions of varying concentrations. Ordinate and abscissa as in Fig. 3. The curves are marked to show the molar concentration of acetyl choline.

A partial recovery of activity on the part of tissues exposed to a constant concentration of drug has been observed by the author in the case of other drugs acting on the frog's heart, and also has been described as a feature of the action of adrenaline on a number of tissues. The phenomenon therefore is not peculiar to the case of acetyl choline. I am unable to explain this tolerance effect but my experiments make it improbable that it is due to the entrance of acetyl choline into the cells, as was suggested by Straub.

Another effect which I am unable to explain is that repeated applications of acetyl choline sensitise the heart to the drug. This action is shown by the following figures:

Time since heart isolated	20′	25′	30′	35′	120′
P.c. reduction produced by 10^{-6} molar acetyl choline	72	85	92	94	94

Hearts freshly isolated were found to be less susceptible to acetyl choline than hearts after a few hours' isolation, but it was found very difficult to distinguish with certainty between effects due to prolonged isolation and those due to repeated application of the drug.

In practice errors due to these causes were avoided by always ignoring the first few responses to acetyl choline given by a heart. A similar sensitisation of the heart after repeated administration of a drug, or after prolonged perfusion, occurs with other drugs and therefore this effect is not peculiar to acetyl choline.

Individual variations in sensitivity to acetyl choline. The response of frogs' hearts to acetyl choline is characterised by a remarkable individual variation. Experiments made upon 71 hearts gave the following results:

TABLE IV

Molar concentration of acetyl choline needed to produce 50 p.c. reduction in response		3×10^{-5}	3×10^{-5} to 3×10^{-6}	3×10^{-6} to 3×10^{-7}	3×10^{-7} to 3×10^{-8}	3×10^{-8} to 3×10^{-9}
Number of hearts	(1) Immersed ventricular strip	0	5	22	12	9
	(2) Isometric ventricle	2	0	2	11	7

In the first series the ventricular strip was immersed in the fluid and hence, for reasons already mentioned, the drug produced a more powerful action with the second than with the first method, but in both cases the figures show that the sensitivity of the hearts varies over at least a thousandfold range of concentrations.

In addition to this type of variation in sensitivity to acetyl choline the hearts also varied as regards the maximum effect that could be produced by the drug. In all cases the relation between concentration and action resembled that shown in Fig. 2. A concentration about one hundred times that needed to produce a 50 p.c. reduction produced an almost maximum action, and little further effect was produced however much further the concentration of the drug was raised. The maximum reduction produced was in most cases more than 90 p.c. of the normal beat, but in a few cases lower figures were obtained, and occasionally 60 p.c. reduction was the maximum effect that could be produced, even when the concentration of the drug was increased to several thousand times that sufficient to produce a reduction of 50 p.c.

I am unaware of any other case in which the natural susceptibility

of a tissue to a drug shows a similar range of variation. As far as I could determine the sensitivity of the hearts were not influenced by the season or by the sex of the animal. There are a large number of possible experimental errors that might cause apparent variation in susceptibility, but great care was taken to exclude all possible errors and the variation was found to continue unaltered.

Discussion. The experiments show that acetyl choline is rapidly destroyed by the frog's heart, and that this destruction must occur within or on the surface of the cells, since the ferment does not diffuse out into fluid kept in the heart. The relation between the destruction of acetyl choline and its concentration follows the usual adsorption formula. These facts suggest that the drug is adsorbed on the heart surface and there destroyed. Experiments made to measure the amount of drug adsorbed by the heart failed to show any certain relation between the amount adsorbed and the amount destroyed, but these experiments were of necessity subject to large experimental errors.

My experiments suggest that at least two independent processes occur when acetyl choline is brought in contact with tissues: firstly, an adsorption and destruction of the drug by the tissues, and secondly, a reaction between the drug and certain specific receptors. The latter process which produces the specific action is probably a reaction with receptors on the surface of the cells. The fact that fixation of a drug by cells can proceed independently of its specific action was shown by Cook(8) in the case of methylene blue acting on the frog's heart.

The action appears to be completely reversible since a similar effect can be produced and removed by washing out a hundred or more times on the same heart. Nevertheless, a certain amount of irreversible change occurs for the heart's sensitivity is permanently increased by the first few applications of the drug. On the other hand, when the heart is left in contact with the drug it establishes a certain degree of tolerance because the initial effect produced decreases after a few minutes. These facts indicate that the factors influencing the response of the heart to acetyl choline must be complex.

The curve relating the action of acetyl choline with the concentration of the drug can be explained most simply on the assumption that a freely reversible monomolecular reaction occurs between the drug and a limited number of receptors of uniform sensitivity.

Gaddum(9) found that the relation between the concentration of adrenaline and its action on the rabbit's isolated uterus followed a curve similar to the one shown in Fig. 2, and suggested that the relation might

be due to a frequency effect. The relation cannot be explained simply in such a manner, but it can be interpreted on these lines by assuming that the drug acts on a population of receptors that vary in sensitivity, and that this variation is of an extreme "skew" type such that the distribution of receptors plotted against the logarithm of their sensitivity to the drug gives a distribution curve of the usual type. This hypothesis is mentioned because it would accord with the remarkable variation in the response of individual frogs to acetyl choline shown in Table IV.

Section II. The influence of ions on the action of acetyl choline.

It is well known that the actions both of para-sympathetico-mimetic and of sympathetico-mimetic drugs are influenced by the ionic concentration of the milieu of the tissue upon which they act. This fact, coupled with the resemblance between the effects of vagal stimulation and of excess of potassium, has led to numerous speculations relating vagal action with the action of ions.

Unfortunately the evidence regarding the influence of ions on vagal action in the frog is conflicting. Some of this confusion may be due to the fact that, as shown by Ten Cate(10), the effects produced by the sympathetic and the vagus on the frequency of the frog's heart are not influenced by ionic changes in the same way as are the effects on the force of contraction of the heart. In this paper only effects upon the force of contraction will be considered.

There is a fairly good agreement that the vagus is paralysed by complete lack of potassium (Ten Cate(10)), and by complete lack of calcium (Ten Cate(10), Busquet and Pachon(11), Hagan and Ormond(12)), although Brine(13) denies this latter effect. Such a paralysis does not denote any specific action of these ions on the vagus because ionic changes of this extent also paralyse other nerve endings.

The chief evidence regarding the effect of slighter changes in ionic concentrations is as follows.

Loewi(14) and Kolm and Pick(15) state that the vagal excitability is increased when the calcium concentration is diminished but Asher(16) considers the evidence on this point to be doubtful.

Ten Cate(10) found that excess of calcium antagonised the action of the vagus, although Howell(17), Loewi(14) and Cori(18) found that this ionic change produced no certain action. Zwaardemaker and Lely(19), Asher(20) and Ten Cate(21) all agree that lack of potassium first augments and finally abolishes the action of the vagus. Excess of

potassium has a doubtful action. Ten Cate(10, 22) and Burridge(23) state that it diminishes the vagal action, but some of Ten Cate's figures(10) suggest the reverse effect. Reduction in the sodium chloride content paralyses the vagus (Witanowski(34)). Finally, Andrus(25) found that vagal stimulation produced a greater action on the tortoise heart in neutral than in alkaline (*p*H 8·0) Ringer.

The evidence is equally uncertain regarding the influence of ionic changes on the action of vago-mimetic drugs.

Excess of calcium antagonises muscarine on the frog's heart (Zondek(27), Loewi and Ischisaka(28)), although Loewi(14) had previously denied this, and Bouchaert(26) found that it did not affect the action of pilocarpine. Excess of calcium also antagonises acetyl choline. (Kolm and Pick(15) on frog's heart; Voss(29) on frog's vessels.)

Bouchaert(26) found that lack of potassium inhibited the action of pilocarpine on the frog's heart, and Voss(29) found that excess of potassium augmented the action of this drug on frog's vessels.

Witanowski(34) found that reduction in sodium chloride slightly reduced the action of acetyl choline on the frog's heart.

Andrus(25) found that acetyl choline produced a greater action on the rabbit's auricle at a *p*H of 7·0 than at a *p*H of 8·0. The author(1) stated that the effect of acetyl choline on the frog's heart was unaltered by changes in the reaction, but this conclusion, I have since discovered, was due to a technical error, for the experimental method did not ensure that the heart cells were bathed sufficiently thoroughly with fluid to demonstrate properly the effects of changes in reaction.

Voss(29) found that acetyl choline acted more powerfully on the frog's heart in alkaline solutions than in neutral solutions.

This summary of results shows the variety of opinions that exist regarding the influence of ions on the action of the vagus and of vago-mimetic drugs. Part of the confusion is due to the fact that the vagus, like other nerves, is paralysed by a large excess of potassium or by complete lack of calcium. The effects of such extensive changes cannot therefore be compared with the effects of moderate changes in ionic content. Even allowing for this fact the evidence is too conflicting to provide any certain conclusions.

Experimental methods. The methods described previously in this paper were used. In most cases the effects of changes in ionic content were determined on the isometric ventricle of the frog, using 10 c.c. of fluid. Ventricular strips irrigated with jets of fluid were used to determine the effect of change of reaction, since in this case it was important

to wash away rapidly from the cell surfaces any carbon dioxide that was formed. Ringer's fluid with a phosphate buffer was used for neutral or acid fluids, and borate was used as the buffer for alkaline fluid.

Calcium concentration and action of acetyl choline. Variations in calcium concentration affect the force of contraction of the frog's heart profoundly and this effect is even more apparent with isometric than with isotonic records. The normal Ringer contained one millimolar calcium, and reduction of this to one-quarter reduced the response about 90 p.c., whilst increase to two millimolar about doubled the force of contraction; further increase in calcium concentration produced little further increase in the force of contraction.

Table V shows that increase of the calcium content of the Ringer above normal reduces the sensitivity of the heart to acetyl choline, but that reduction of the calcium content below normal does not alter this sensitivity. The response of the heart when the calcium content was below 0·5 millimolar was so feeble that it was not possible to measure accurately the effect produced by acetyl choline, but no striking change in sensitivity was noted under these conditions.

TABLE V.

Molar calcium content $\times 10^3$		0·5	1	2	4
Molar conc. of acetyl choline $\times 10^3$ required to produce 50 p.c. reduction in response of frog's ventricle	(1)	8	8	—	40
	(2)	2·5	2·5	6·4	—

Although alterations in the calcium content produced considerable alterations in the sensitivity of the heart to acetyl choline, yet the relation between the concentration of acetyl choline and the action produced always followed the formula given with Fig. 2 and the effect of changes in calcium concentration was simply to alter the constant K.

The antagonism between acetyl choline and calcium excess is in accordance with most of the observations made regarding the effect of this ionic change upon the action of vago-mimetic drugs.

TABLE VI

Molar conc. KCl $\times 10^3$	0·5	1·0	2·0	4·0	8·0	12·0
Isometric response as p.c. of normal	188	150	100	77	36	6

Influence of potassium on the action of acetyl choline. The normal Ringer contained two millimolar potassium, and the effect of changes in this concentration are shown in Table VI. These results agree fairly well with figures that the writer previously has obtained with the ventricular strip method (30). The effects produced by changes in the potassium concentration on the response of the heart to acetyl choline

are shown in Table VII. This shows that reduction in the potassium concentration increased the sensitivity of the heart to acetyl choline whilst increase in the potassium concentration decreased this sensitivity.

TABLE VII.

Molar content of potassium $\times 10^3$	0·5	1·0	2·0	4·0
Molar conc. of acetyl choline ($\times 10^3$) which produced 50 p.c. reduction in response	1	3	5	8

Increase in the potassium chloride content above 0·004 molar produced so great a reduction in the force of contraction that it was difficult to measure the effect of acetyl choline, but no striking change in sensitivity to acetyl choline was observed with these higher concentrations of potassium. The fact that decrease in potassium increases the action of acetyl choline accords with the results of various workers who have shown that this change increases the sensitivity of the frog's heart to vagal stimulation.

The influence of reaction on the response to acetyl choline. Changes in hydrogen ion concentration produced a very marked effect on the isometric response of the whole ventricle. The carbon dioxide produced by the heart cells was a possible source of error since it tended to alter the reaction of the fluid in contact with the cells; therefore experiments were also made with ventricular strips irrigated with a jet of fluid. The two types of experiments gave concordant results and typical figures are shown in Table VIII.

TABLE VIII

Hydrogen ion concentration $\times 10^9$	0·1	1·0	6	30	60	300	600
Isometric response in cm. water	10	60	40	28	19	10	2

Experiments both with the strip method and with the whole ventricle showed that decrease in the hydrogen ion concentration antagonised the action of acetyl choline. Typical results are shown in Table IX.

TABLE IX.

Hydrogen ion concentration	10^{-7}	$1{\cdot}6 \times 10^{-6}$	10^{-5}
Molar concentration of acetyl choline $\times 10^9$ producing 50 p.c. inhibition	2	3·2	8

Accurate results could not be obtained with solutions with a *c*H above 10^{-7} because the heart beat feebly in such solutions, but the experiments showed that acidity did not alter the action of acetyl choline to any striking extent.

These results confirm the conclusions of Andrus(25) (acetyl choline on rabbit's auricle) but are opposed to those of Voss(29) (acetyl choline on frog's heart).

The figures given above do not show adequately the full changes produced by alkalinity on the response to acetyl choline. Not only does the heart become less sensitive as measured by the concentration needed to produce 50 p.c. of maximum inhibition, but also the maximum inhibition that can be produced by acetyl choline is reduced.

This difference is shown clearly in Fig. 5, which shows that when

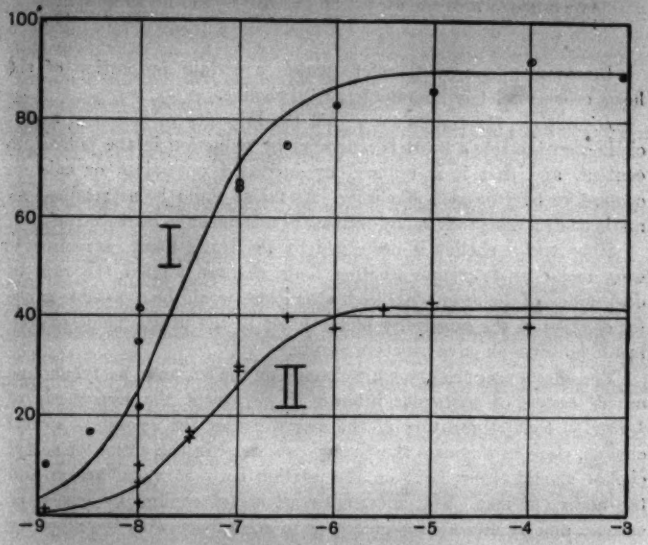

Fig. 5. Influence of reaction on the action of acetyl choline.

Ordinate and abscissa as in Fig. 2.

The crosses show the figures observed, whilst the curves were drawn to the following formulae. (x and y as in Fig. 2.)

Curve I. Phosphate buffer pH = 7·5. Formula $k.x = \frac{y}{90 - y}$. (k = 40,000,000.)

Curve II. Borate buffer pH = 9·0. Formula $k.x = \frac{y}{42 - y}$. (k = 20,000,000.)

the heart was perfused with Ringer pH 7·5 the maximum effect produced by acetyl choline was a reduction of 90 p.c. in the response, but that when the pH was 9·0 acetyl choline only produced a 40 p.c. reduction in the response.

Figures from other experiments showing the same effect are given in Table X.

TABLE X

Hydrogen ion concentration	Maximum reduction in response produced by acetyl choline					
	(1)	(2)	(3)	(4)	(5)	Average
10^{-7} to 3×10^{-8}	100	95	90	95	—	95
$1{\cdot}6 \times 10^{-8}$ to 6×10^{-8}	65	92	50	90	90	77
10^{-8}	—	80	50	—	42	57

Increased alkalinity therefore makes a certain proportion of the heart cells completely immune to acetyl choline.

Discussion. My results show that the intensity of action of acetyl choline on the frog's heart is increased by reduction in the potassium content, and that it is reduced by increased potassium or calcium content or by increased alkalinity. As far as could be ascertained no marked effect was produced by decrease of calcium or by increased acidity.

Since acetyl choline is destroyed by the frog's heart, experiments were made to determine whether ionic changes altered the rate of destruction of the drug, since such alterations would be a possible cause for changes in the sensitivity of the heart to the drug. These experiments however all gave negative results.

The effects produced by ionic changes on the action of acetyl choline are of course of particular interest if we accept the hypothesis of Loewi(2) that stimulation of the vagus causes the release of acetyl choline, since in this case the changes observed in the action of acetyl choline should throw light on the relation between vagal action and the action of ions. The antagonism of acetyl choline by excess of calcium and by increased alkalinity is in accordance with the majority of observations regarding the effect of these changes on vagal action. These effects are moreover such as would be anticipated because excess calcium and increased alkalinity produce effects on the frog's heart almost exactly the opposite of those produced by either acetyl choline or by vagal stimulation. The effects produced by alterations in the potassium content on the action of acetyl choline are much more difficult to understand.

Diminution of potassium concentration definitely increased the action of acetyl choline, and several observers have shown that this ionic change also increases the action of the vagus. Increase in potassium content antagonised the action of acetyl choline, but the evidence regarding the influence of this change on the vagus is indecisive.

These effects are the opposite of those that might have been anticipated. In the first place excess of potassium depresses the contractility of the heart in a manner very similar to vagal stimulation, whilst lack of potassium produces a systolic effect somewhat resembling sympathetic stimulation. In the second place calcium and potassium are supposed to be antagonists and yet variations in these two ions produce a similar effect on the response to acetyl choline or to vagal stimulation. Calcium and potassium are therefore not antagonists as regards their effects on the response of the heart to acetyl choline. Other evidence is available showing that calcium is an imperfect antagonist of potassium. For example, calcium lack and potassium excess produce different changes in the electrical response of the frog's heart (Daly and Clark(31)), and on conduction in the tortoise auricle (Seliskar(32)). Moreover changes in the content of one ion can only be antagonised as regards their action on contractility over a fairly small range of concentrations (Clark(30)).

The effects produced by ionic changes on the response of the heart to acetyl choline and to vagal stimulation can be explained on the hypothesis that these latter actions are dependent upon the perfusion out from the heart of potassium. Howell and Duke(33) showed that vagal stimulation liberated potassium from the mammalian heart. This was denied by Hemmeter(34) who worked with elasmobranch hearts, but was confirmed by Asher(20) who used the frog's heart.

The passage out of potassium from the heart should be antagonised by excess of potassium in the Ringer and should be favoured by lack of potassium. The writer(35) has shown that perfusion of the frog's heart with potassium free Ringer results in a loss of potassium from the cells.

On the other hand, this loss of potassium would be antagonised by any change that made the cell wall less permeable, and excess of calcium and increased alkalinity are believed to produce this effect. Hence this hypothesis would explain why the two last mentioned ionic changes antagonise acetyl choline. Unfortunately this hypothesis is very difficult to reconcile with the fundamental fact that lack of calcium, excess of potassium and acetyl choline all produce a very similar depression of the contractility of the frog's heart.

My experiments support Ten Cate's(10) conclusion that the action of the vagus or of vago-mimetic drugs cannot be identified with the action of potassium in any simple manner, but that nevertheless there appears to be some special connection between vagal action and the distribution of potassium in the heart cells and in the surrounding fluids.

Conclusions.

Part I.

1. The relation between the concentration of acetyl choline and its action on the frog's heart has been tested with a variety of experimental methods and in all cases the same relation has been found as was described in a former paper.

2. The destruction of acetyl choline by the frog's heart resembles a ferment action and the relation between the amount destroyed in unit time by unit weight of tissue (x) and the concentration (c), over a range of concentrations from 10^{-3} to 10^{-6} molar, is given by the formula $K.c^{1/n} = x$. ($K = 7 \times 10^{-6}$ and $n = 1{\cdot}2$).

3. Acetyl choline combines with or can be washed out of the heart in a few seconds, and the rate of action and rate of wash-out are of a similar order.

4. The presence of acetyl choline cannot be demonstrated within heart cells after prolonged exposure to the drug.

5. The amount of acetyl choline that produces the specific action of the drug is probably considerably smaller than the maximum amounts previously calculated.

6. The individual susceptibility of frogs' hearts to acetyl choline varies over a remarkably wide range.

Part II.

The action of acetyl choline on the frog's heart is modified by changes in the ionic content of the Ringer's solution.

The chief changes produced are as follows:

The action of acetyl choline is reduced by increased calcium or potassium content or by increased alkalinity.

The action of acetyl choline is increased by decreased potassium content.

The expenses of this investigation were in part defrayed by a grant from the Government Grants Committee of the Royal Society.

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FURTHER OBSERVATIONS ON THE REACTION OF SMOOTH MUSCLE TO THE H-ION CONCENTRATION.

By B. A. McSWINEY and W. H. NEWTON.

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In a previous paper(1) the effect of changes of *p*H upon smooth muscle capable of sustained variation in length was described and the length of the muscle was shown to be dependent on the *p*H of the surrounding fluid for any given period. Differences in direction and sensitivity of the response at different ranges of the *p*H scale were also demonstrated. In this paper experiments are described which deal with the effects of similar changes of *p*H on strips of smooth muscle which normally exhibit rhythmic activity with little or no tonus mechanism.

Farndon(2) in 1908 stated that with the mammalian uterus preparation alkali augmented tonus with diminution of spontaneous contractions and death in contraction. Acid on the contrary lowered the tone of the muscle, with diminution of spontaneous contractions; sudden strong acidity causing death in forcible contraction. Young(3), in 1914, found that HCl up to 0·006 p.c. caused relaxation of the muscle of the small intestine as did also CO_2, but as phosphates were present in the solution it is difficult to calculate the *p*H corresponding to the strength of the solution. 0·5 p.c. HCl was found to abolish all movements. Botazzi(4) in 1916 stated that the tone of isolated intestine was increased by all alkalies and diminished with acid. Evans and Underhill(5), in 1923, showed that the effect of a small increase in the *p*H was to augment the frequency of contractions while a decrease in the *p*H caused a diminution in the rate.

Method. Strips of muscle mainly from the region of the pylorus and lower body of the cat's stomach were used in these experiments. The regions proximal to the antrum, while exhibiting rhythmic movement were also capable of permanent change in length, and were therefore unsuitable for a study of effects dealing primarily with alteration of rhythm. The strips of muscle were suspended in Ringer-Tyrode solution in the glass muscle chamber. Phosphates were omitted, as in previous experiments,

to obviate precipitation in alkaline solution and also to have sodium bicarbonate as the sole buffer. To control the *p*H of the solution, O_2 and CO_2 were bubbled in by the methods previously described. For large alterations of *p*H sodium hydroxide and hydrochloric acid were used. The temperature of the fluid was controlled at 37° C. by a thermostat.

Experimental results. The effect of a moderate fall in *p*H was to cause a decrease in the frequency of the spontaneous contractions of the muscle from the pyloric antrum with no observable change of the base line (Fig. 1). Occasionally this result was accompanied by a diminution in height of the contractions; often, however, the contractions showed a preliminary increase in size which was, in many experiments, sustained. A greater decrease in the *p*H caused a cessation of the rhythmic contractions and the tracing became a straight line.

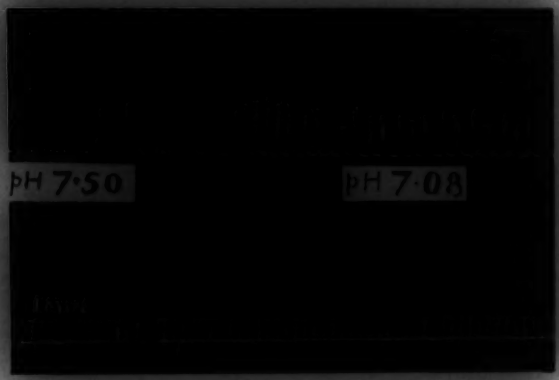

Fig. 1. Antrum of cat. Tracing to show decrease of rate of contractions on decrease of *p*H with onset of spasm. Time tracing, one minute.

A moderate increase in *p*H caused acceleration of the contractions which were often increased in height. If the *p*H of the solution was raised above *p*H 8 the amplitude of the movements tended to become smaller though the rate of contractions showed a further increase. No alteration in the base line was observed throughout this range.

In most experiments an interesting type of contraction was observed when the rhythm of movement was depressed by increasing the acidity. Two or more contractions became grouped together, the lever making

an incomplete return to the base line between phases. The groups of contractions occurred quite regularly and, in tracings taken from preparations of antrum, replaced the single contractions. This spasm effect is seen in Fig. 1, being initiated as the *p*H is lowered. In Fig. 2 the effect may also be observed passing off as the *p*H is raised. Spasm usually occurred in the region of *p*H 7·1.

Fig. 2. Circular preparation of cat's antrum. Tracing to show augmentation of rate of contractions with disappearance of spasm on increase of *p*H. Time tracing, one minute.

In regions of the stomach other than the antrum, *e.g.* lower body, similar effects were observed in the main, but the results were complicated by the simultaneous occurrence of tonic changes, accompanied by irregularities in the contractions similar to those described in our last paper. Almost invariably a decrease in frequency was accompanied by an increase in amplitude and vice versa. The final abolition of contractions, by increase in acidity, was preceded by spasm, but like the contractions themselves, this stage was more irregular than that seen in the antrum (Figs. 3 and 4). An initial spasm was often seen on lowering the *p*H and once or twice violent spasm occurred for no apparent reason. It may be recalled that, in the fundic region of the greater curvature of the cat, a peculiar tonus effect was observed on lowering the *p*H, a cycle of relaxation and contraction preceding the main relaxation. In the present series of experiments the base line, instead of falling when the *p*H was diminished,

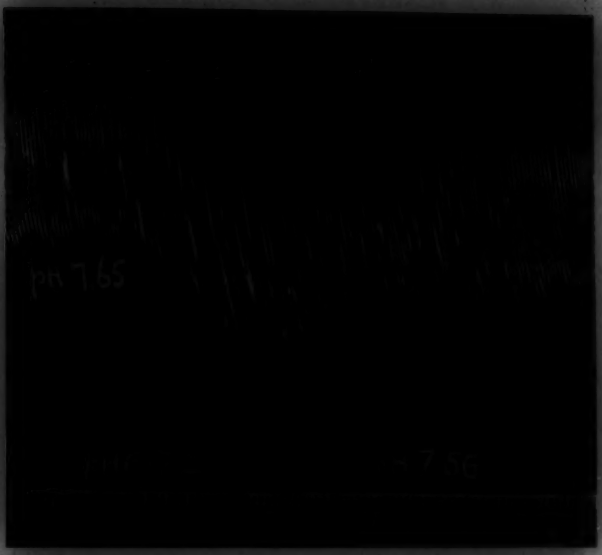

Fig. 3. Preparation of sling of cat. Tracing to show effect on the length of the muscle and rate of contractions of alterations of pH. Time tracing, one minute.

Fig. 4. Upper body of cat. Tracing to show onset and disappearance of spasm. Time tracing, one minute.

rose steadily and fairly rapidly for five or six minutes, while the rate of contractions was well maintained. After this initial effect the base line began to fall in the usual way, the frequency of contractions becoming much diminished with an accompanying increase in the amplitude.

In the description of our experimental results we have sharply divided the antrum from the remainder of the stomach in order to separate the simple antral type of change from the more complicated reactions of the proximal regions. There is, however, a gradual transition from one part to another, as even from circular and longitudinal strips of the fundus, tracings have been obtained which show a fair degree of

Fig. 5. Antrum of cat. Tracing to show response of muscle to large decrease of pH. Time tracing, ten minutes.

regularity in the rate and height of contractions. The general rule is that as the fundus is approached, the rhythm becomes gradually more irregular and the muscle fibres are more prone to alter in length as the *p*H is varied. This is accompanied by a greatly increased frequency of spontaneous contractions, in contrast to the slow regular pulsations of the antrum.

When the muscle has been stimulated by pilocarpine, the effects of *p*H changes upon the rhythm can still be superimposed. Examination of the tracings shows acceleration on decreasing the acidity, retardation on increasing the acidity, spasm and total cessation can also be observed. The effects are, however, damped by the initial treatment of the muscle and delicate transition effects are difficult to obtain.

The reaction of rhythmic strips of the antrum to large changes in *p*H are interesting. As in previous experiments, the action of the strong acid was ascertained by adding to the 250 c.c. of Ringer-Tyrode solution, which bathed the muscle, successive doses of 0·125 c.c. of 30 p.c. HCl. The first addition of acid caused a complete cessation of all spontaneous contractions: the second, a rise in the base line: the third, a further rise in the base line while subsequent doses have no effect except possibly a fall of base line.

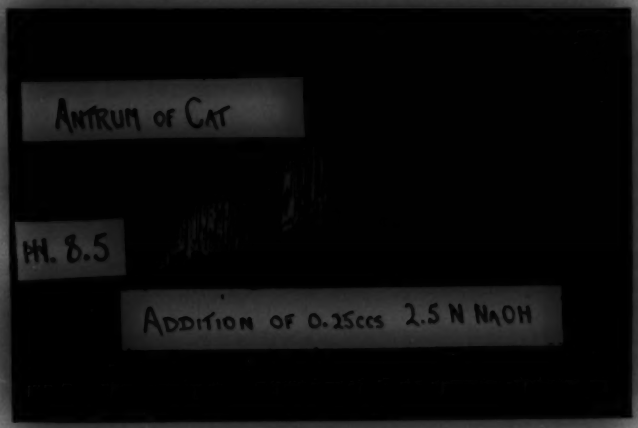

Fig. 6. Antrum of cat. Tracing to show response of muscle to large increase of *p*H. Time tracing, ten minutes.

In order to obtain any response of the tissue on addition of 2·5 *N* NaOH, beyond an alteration in the rate of contractions, it was found necessary to cut off the stream of CO_2, to prevent the formation of bicarbonate. The solution has, therefore, an initial *p*H of 8·5–9·0, the oxygen bubbling being continued throughout the experiment. In experiments in which the initial value of the H-ion concentration of the solution was regulated to a value of *p*H 7·5 by the use of CO_2, both gases being left bubbling, the rhythmic contractions were increased in frequency, but the strip showed no alteration in length, even when the total alkali in the solution equalled *N*/20.

If the bubbling of CO_2 was arrested the addition of 0·25 c.c. of 2·5 *N* NaOH, first stimulated then depressed the spontaneous contractions. The stimulation was sufficiently great to initiate contractions in strips of quiescent antral muscle (Fig. 6). A rise of base line accompanied the rhythmic changes but was not marked until a concentration of *N*/100 NaOH had been reached (1 c.c. of 2·5 *N* NaOH to 250 c.c. of solution). At this point, a marked sustained contraction always occurred, usually with cessation of rhythmic contractions. Further addition of alkali failed to bring about the end relaxation such as was obtained in the muscle of rabbit fundus.

Discussion.

We are able to divide smooth muscle into three divisions according to its movements and tonus: (1) muscle capable of only change in length; (2) muscle showing rhythmic activity; (3) muscle capable of changes in length and rhythmic movement. These three types of muscle can be demonstrated in the stomach.

In a previous set of experiments we showed that the first type of muscle responded to changes of *p*H in a manner similar to the swelling of gelatine on changing the H-ion concentrations. In these experiments the effect of alterations of *p*H on the rhythmic type of smooth muscle has been observed. We find that moderate decrease of *p*H decreases the rate and amplitude of movement, but causes no change of base line. Addition of strong acid ultimately brings about contraction of the muscle. The response of the muscle to alterations of *p*H in the opposite direction are similar; moderate increase causes increase in rate of contraction but no change of length; further addition of strong alkali, however, brings about a contraction of the muscle.

On decreasing the *p*H a grouping of contractions was frequently observed in the region of *p*H 7·1. We have termed the condition "spasm"

owing to the similarity of the tracings to those obtained by McCrea and McSwiney(6). These observers in recording the movements of the pyloric antrum on stimulation of the peripheral end of the vagus nerve found that "a strong stimulus may cause a sustained contraction, the rhythmic movements increasing in rate and starting above the base line indicating summation." The tracings illustrating this statement are similar to those obtained in these experiments. It is of interest that stimulation of the vagus with a strong current can bring about a similar condition to that obtained on alteration of the H-ion concentration.

The reaction of the antral muscle to changes in H-ion concentration may be summarised as (1) alterations of rhythm to moderate changes; (2) alteration in length to extreme changes. If we compare these results with those obtained on the fundus, we find that the sustained contraction occurs on the acid side at the same point *p*H 5·9 and at a comparable point on the alkaline side.

Changes in length of the fundus muscle by small alterations in *p*H to either side of *p*H 7·5 correspond to the alterations in frequency of contractions found in the tracings taken from strips of the pyloric antrum. The contractions obtained by addition of strong acid and alkali appear to be similar in all types of tissue. These findings are not in entire agreement with the results of Evans and Underhill who found that smooth muscle always relaxed on the addition of acid but in their experiments a muscle possessing considerable tonus was used which would correspond to a strip from the lower body of the stomach.

We do not wish to suggest that these results are any indication of a lactic acid mechanism in smooth muscle, as the response of the tissues under the conditions of experiments appears to us to be of the nature of a protein reaction, similar to the swelling of gelatine, as we have previously pointed out. The variation of frequency to alteration in H-ion concentration occurs in exactly the same range as the reversible change in length recorded in "tonus" muscle. The concentrations at which the sustained contraction takes place are also comparable. The different type of response occurring in strips of smooth muscle taken from different situations suggests that the tissues possess different buffering properties and this property may be related to function. The reaction of the fundic muscle (tonus muscle) to alterations in *p*H is a graded reaction throughout, while in pylorus muscle the comparable protein reaction is represented by the end contraction which occurs outside physiological limits.

To test this hypothesis, the reaction was studied in other preparations, whose structure contains, at the most, only a small amount of smooth

muscle. The tracing in Fig. 7 illustrates the effect of changes in H-ion concentration on a strip of human skin. In this tissue a reaction only

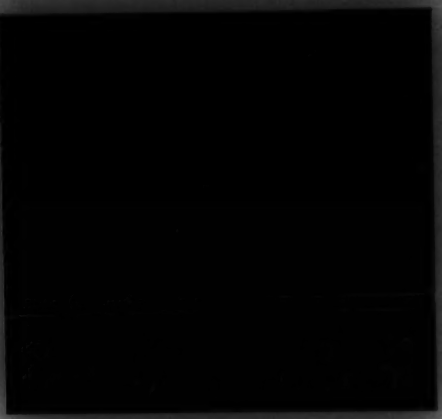

Fig. 7. Strip of skin. Tracing to show effect on tissue of changes of *p*H. Time tracing, one minute.

occurred with large changes of *p*H; the response was, however, similar in direction to the results obtained with strips of smooth muscle under similar conditions. An attempt was made to demonstrate a pure protein effect on a strip of gelatine suspended in cold solution (Fig. 8). A change of length was obtained on addition of strong acid and strong alkali as with skin, but the reaction was opposite in direction, relaxation on decrease and contraction on increase of *p*H. The change in length occurs at a H-ion concentration, at which, according to Loeb(7), gelatine increases in volume. The reaction may be explained on the suggestion that the increase in the size of the gelatine, under the conditions of tension, would be represented on the tracing by an increase in length, whereas in the muscle prism, swelling of the cell, resulting in an increase of pressure, would cause a bulging of the lateral wall and a shortening of the whole element.

In view of the suggestion that the reaction of smooth muscle to alterations in H-ion concentration is of a protein nature, a recent paper by Gorter and Grendel(8) is of interest. These observers demonstrated the effect of H-ion concentration on the thickness of a protein film. At

the iso-electric point and at a *p*H 1 or 2, a very thin film is obtained, but at both sides of the iso-electric point the thickness is far greater.

Fig. 8. Strip of gelatine. Tracing to show change of length on alteration of *p*H. Time tracing, one minute.

The thickness of these films is often about three times greater than the monomolecular. The curve, showing the relation of the *p*H to the thickness of a casein film, is similar to the curve we have previously published.

Conclusions.

1. The reaction of rhythmic contracting smooth muscle to changes in the H-ion concentration is analogous to the reaction of "tonus" muscle.

2. Alterations in frequency of contractions, augmentation on increasing, and diminution on decreasing the H-ion concentration, within narrow limits, are analogous to the changes in length observed in "tonus" muscle.

3. Sustained contraction of both types of muscle was observed with either a large increase or decrease of H-ion concentration at comparable points.

4. A grouping of contractions (spasm) was observed on decreasing the *p*H.

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VASCULAR PROPERTIES OF TRAUMATISED AND LAKED BLOODS.

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Attempts to demonstrate the presence of a vaso-dilator substance in the venous blood collected from the limb during the period of reactive hyperæmia following obstruction of its circulation have led to observations of vaso-dilator action of shed blood which form the basis for this report. One of the most plausible theories for the explanation of reactive hyperæmia, advanced by Anrep(1) and supported by Lewis and Grant(2) and Goldblatt(3), is that a vaso-dilator metabolite is formed in the tissues of the limb during the period of vascular obstruction, and that this causes relaxation of the capillaries and arterioles. Such a substance would, after release of the obstruction, be either destroyed *in situ* or carried away by the circulating blood.

Dr G. V. Anrep, at whose incentive the work was begun, suggested that circulation of the venous blood collected from the limb during the period of reactive hyperæmia might produce vaso-dilatation, and devised a vivi-perfusion apparatus for that purpose.

Description of the apparatus. The apparatus consists of a flask attached to a cannular system which can be inserted in the course of the main artery of the limb (Fig. 1). By adjusting the T-bore tap (*S*) the blood may be sent directly from the proximal into the distal limb of the cannula or it may be diverted through the flask (*F*) passing in at one end and out at the other by way of the connecting tubes (*C* and *D*). For purposes of filling or emptying, the flask is provided with extra outlets at the top and bottom. Before emptying the flask the outlet tube *D* is obstructed by turning its stopcock, or, in the absence of a stopcock, by the application of a clamp. A tube for registering the arterial pressure connects the distal limb of the cannula with a mercury manometer. The bore of the glass tubing leading to the flask is 4 mm. and that of the glass cannulæ which are inserted in the artery is the largest that can be introduced, averaging 2½ mm. for the femoral artery of the dog. Flasks varying in size from 30 c.c. to 75 c.c. were used.

They were of Dewar construction in order to reduce the loss of heat from the blood.

Dogs weighing preferably 10 to 15 kg. were used; 0·3 to 0·5 c.c. of 10 p.c. morphine acetate was given hypodermically. In the early experiments anæsthesia was induced by ether and continued by chloralose, 0·075 grm. per kg. body weight, given intravenously. This frequently produced slow and deep respirations which disturbed the plethysmographic tracings. Urethane 0·3 grm. per kg. was then used in place of chloralose with improved results but respiratory difficulty was sometimes encountered. Finally ether was given alone by means of an automatic inhalation apparatus and with its use the most uniformly satisfactory tracings were obtained. A 3-inch incision with its middle point at Poupart's ligament is made over the vessels of the left lower limb. The upper part of the femoral artery and the lower part of the iliac artery are exposed and all of their branches ligatured and divided. The peritoneum is retracted upward and the posterior iliac artery ligatured. The branches of the femoral vein are then tied off and severed. The limb is denervated by division between ligatures for hæmostasis of the anterior crural and sciatic nerves and of the branches of the lumbar plexus along the iliac vessels. 0·35 to 0·4 grm. of heparin is then given intravenously to prevent coagulation of the blood. A Y-cannula is inserted into the femoral vein and the cannulæ of the vivi-perfusion apparatus into the femoral artery. (In later experiments where arterial blood only was used no cannula was put into the vein.) The flask and T-bore stopcock are supported by clamps from a ring stand. Connections are then established for recording blood-pressures in the carotid artery and in the cannula of the femoral artery. A 5-second time marker is used.

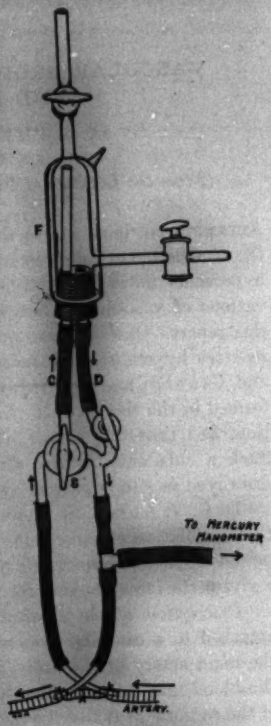

Fig. 1. Anrep's vivi-perfusion apparatus.

A plethysmograph is applied to the limb and filled with water at a temperature of 35°. Electric light bulbs or heaters are applied alongside the flask and plethysmograph to maintain constant temperatures and the animal's temperature is kept normal by the use of blankets and by the application of heat externally when necessary.

Under favourable conditions the experiment thus set up may be carried on over a period of 4 to 6 hours before clotting occurs or the vessels lose their power to respond. Altogether 92 experiments have been performed with this apparatus and its modifications, with varying degrees of success in 69.

Results of experiments.

Obstructions of the artery were interspersed with the vivi-perfusion experiments in order to test the hyperæmia reactions (Fig. 3 *a*). In general they were found to correspond with those reported by Goldblatt. A 30 c.c. flask was used regularly, except when specified. 30 c.c. of blood were collected from the femoral vein immediately after the release of obstructions of the femoral artery varying from 1 to 5 minutes in duration and transferred to the flask. It was circulated in the limb 1 to 5 minutes after recovery from the hyperæmia and regularly produced vaso-dilatation as evidenced by increase in limb volume and decrease in limb pressure lasting over a period of 40 to 60 seconds. Samples of blood were then collected from the femoral vein after recovery of the limb from the hyperæmia and from the jugular vein, transferred to the flask and circulated in the limb. They were found to produce as marked temporary increase in limb volume and decrease in limb pressure as did the blood collected from the femoral vein during the period of hyperæmia. No special care was taken against traumatism of the blood during these experiments. Arterial blood was stagnated in the flask for 1 to 10 minutes by adjusting the T-bore tap and then circulated in the limb (Fig. 2 *a*). It normally produced no vaso-dilatation and when vaso-dilatation occurred it was less than that produced by the venous bloods.

It was then considered probable that there is normally a metabolite in venous blood capable of producing vaso-dilatation when re-circulated. Since the most marked difference between arterial and venous bloods is found in their gaseous contents, it was thought that decrease in oxygen might be responsible for the reaction. Consequently the effects were determined of both arterial and venous bloods whose gaseous contents were modified outside the body. Blood was placed in tonometers which

were then exhausted by a suction pump for 10 to 15 minutes during shaking. In some instances the blood was then exposed to varying

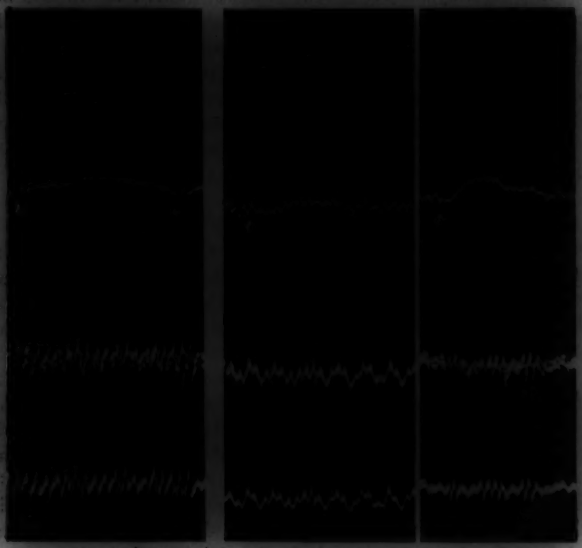

Fig. 2, Exp. 15. *a*. Circulation of arterial blood stagnant in short-circuited flask for 4 minutes. *b*. Circulation of jugular vein withdrawn under hood without shaking. *c*. Circulation of arterial blood removed under hood without shaking.

tensions of CO_2 at 37° and atmospheric pressure. The CO_2 content of the blood was varied in these experiments from minimal values to the largest obtainable by this method. Exhausted blood was also circulated. In all cases, however, and quite independently of all changes made in the blood gases either arterial or venous blood always gave a conspicuous vaso-dilatation when circulated in the limb.

Since traumatism of the blood was a constant factor in all experiments in which the gaseous contents were modified, it was decided to test the influence of traumatism alone. Arterial blood was withdrawn, shaken by hand for varying lengths of time, returned to the flask and circulated. It regularly produced vaso-dilatation (Fig. 3 *c*), the degree

usually varying directly with the duration of the shaking up to a certain point. Active shaking for 10 seconds caused a definite though small

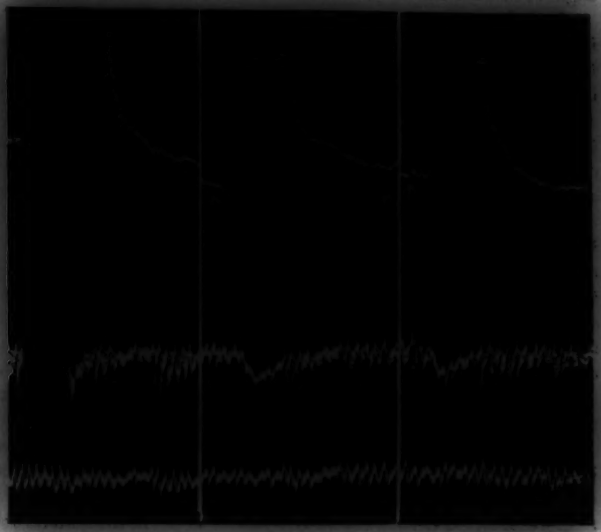

Fig. 3, Exp. 15. *a*. Reactive hyperæmia following obstruction of femoral artery. *b*. Circulation of blood from jugular vein shaken 1 minute. *c*. Circulation of blood from femoral artery shaken 1 minute.

reaction. The reaction increased with prolongation of the period of shaking up to 2 or 3 minutes, when a maximum effect was produced. This corresponded roughly with the length of the obstruction necessary to produce the maximum hyperæmic reaction in the same experiment. Further traumatism for as long as 10 minutes produced no additional effect. Shaking with beads or with a funnel inverted in the beaker into which the blood was withdrawn hastened the rapidity with which the vaso-dilator property developed. Samples of venous blood were subjected to traumatism and were found to produce the same reactions as similarly treated arterial blood (Fig. 3 *b*). In contrast with these experiments it was found that when either arterial or venous

blood was withdrawn for a few minutes and introduced into the flask with care and not traumatised while outside, it usually produced either slight or no vaso-dilator action when circulated in the limb (Fig. 2 *b* and *c*).

In order to determine more accurately the influence of traumatism in the production of the vaso-dilatation, it was decided to traumatise the blood without removal from the flask. A glass bead was inserted in the flask before the start of the experiment. In the course of the experiment blood was trapped in the flask, the rubber tubes leading to the flask were clamped with a hæmostat and then detached from the cannulæ. The flask was then shaken for 1 to 5 minutes and re-attached. Circulation of the blood caused vaso-dilatation which varied directly in degree with the amount of shaking. Circulation of blood in the flasks that had been detached for the same length of time and re-attached without shaking caused either very slight or no vaso-dilatation. This experiment proved that the vaso-dilator property may be acquired by traumatism without exposure of the blood to air. This was further demonstrated when the blood was withdrawn from and returned to the flask under paraffin. If shaken while outside, it caused vaso-dilatation when circulated but it had little or no dilator effect if it had not been shaken.

In order to test for vaso-dilator action of untraumatised venous blood collected from the limb during the period of reactive hyperæmia following the release of arterial obstruction, a cannular system with flask was inserted in the femoral vein as well as one in the femoral artery. With the circulation on the venous side passing through the flask, the artery was obstructed, and then released after 2 to 3 minutes. The venous flask was short-circuited 45 to 60 seconds later, clamped off and transferred to the arterial side. Its blood which had come from the limb during the height of the period of reactive hyperæmia was then circulated, and in numerous trials in four experiments it produced either no vaso-dilatation as shown in Fig. 4 or a very faint vaso-dilatation such as might result from contact of the stagnant blood with the flask during the time in which the test was being carried out. Blood which is stagnant in the flask for more than 5 minutes may acquire slight vaso-dilator properties which increase with prolongation of stagnation. The effect of shaking should be regarded as merely an acceleration of the process.

Light was excluded by the use of a hood and of flasks that were coated with black paint. Blood that was traumatised and circulated with the light excluded caused vaso-dilatation while untraumatised blood did not. The temperature of the blood was determined by the

use of a flask with a bung in the top which held a thermometer. A single-jacket flask holding 40 c.c. of blood was detached after clamping the

Fig. 4. Blood, short-circuited in venous flask at ×, 1 minute after release of 3 minutes' obstruction of femoral artery and, transferred to the arterial side, caused practically no vaso-dilatation when circulated at the arrow point.

rubber tubes, heated or cooled, re-attached and circulated without shaking. Variations in temperature of the blood within 2° to 30° C. had no effect unless the blood was kept out of circulation for several minutes. Traumatised blood, the temperature of which was kept constant, always gave vaso-dilatation.

The hydrogen-ion concentration was determined before and after shaking for 4 to 5 minutes and was found to be either unaltered or very slightly shifted toward the alkaline side. In order to exclude heparin as a possible factor the blood of the dog was defibrinated by withdrawing, whipping, and re-injecting 250 c.c. at a time for 10 or 12 times. The cannulæ were then inserted into the artery without the use of an anti-coagulant. In three attempts the blood coagulated in the tubes before the experiment could be completed. In one successful experiment the circulation continued through the tube for 20 minutes and both arterial and venous bloods were found to produce vaso-dilatation when traumatised before circulation. 0·7 grm. of hidrudin was used in two experiments and the results were the same as those obtained with heparin. Consequently heparin cannot be regarded as a factor in the causation of the vaso-dilatation. Heparinised blood that had not been shaken was centrifugated and the plasma drawn off. When plasma alone was circulated in the limb, it always produced vaso-dilatation. This action may be due to the traumatism during centri-

fugation, or to diminished viscosity. But on the other hand, circulation of the remaining unwashed corpuscles also produced after a temporary decrease in volume a dilatation of the limb. The limb constriction was interpreted as being due to the high viscosity and the dilatation which followed to the vaso-dilator property acquired as a result of the traumatism.

From these experiments it was concluded that blood when traumatised as by shaking for 10 seconds to a few minutes acquires a vaso-dilator property which is independent of exposure to light or air and of changes of temperature and of changes in O_2 or CO_2 content and of hydrogen-ion concentration. All of the reactions described above were also observed in seven experiments in which the sciatic and crural nerves were not cut.

Two theories were entertained as to the nature of the change produced by traumatism of the blood which gives it the vaso-dilator property. One was that a vaso-constrictor substance is destroyed and the other that a vaso-dilator substance is formed or liberated either in the blood or by the tissues when in contact with traumatised blood. The pituitary principle and adrenaline are the two vaso-constrictor substances that have often been held responsible for the maintenance of normal vascular tone. The evidence in favour of either as the important factor is both meagre and fragmentary. Krogh(4) believed, mainly as a result of chemical studies and of perfusion of the vessels of a frog with a dialysate of ox blood, that pituitrin in a concentration of around 1 to 100,000 is the active substance. Dale and Richards(5) regarded it as possibly adrenaline.

From a chemical standpoint both adrenaline and the pituitary principle are far too stable in blood to be destroyed during the few seconds of shaking which are necessary to give it vaso-dilator properties. If, however, this be the case, then it is difficult to imagine that these substances would remain unchanged for several minutes in untraumatised shed blood. Attempts were made to neutralise the vaso-dilator action of traumatised blood by the addition of minute quantities of adrenaline or pituitary extract (B. D. H.) and to find whether the amounts necessary for such neutralisation are of the order which would be expected to be present in the normal circulating blood. When adrenaline was added to 30 c.c. of untraumatised blood in a concentration of 1 to 500 million it was found on circulation that slight vaso-constriction occurred lasting 20 to 40 seconds. 1 to 100 million produced a conspicuous decrease in limb volume and increase in limb pressure.

But when shaken blood was treated with 1 to 500 million of adrenaline the usual vaso-dilatation followed on circulation as if nothing had been added. Even when the adrenaline concentration in the shaken blood was raised to 1 to 200 and 1 to 100 million it still caused the usual amount of increase in limb volume and decrease in limb pressure, but the recovery was more rapid than normal and slight constriction followed. It was found in some experiments that circulation of shaken blood plus as much as 1 in 20 million of adrenaline would at first produce slight increase in limb volume and fall in limb pressure which were followed by rapid and marked decrease in limb volume and slight increase in limb pressure. Unshaken blood to which adrenaline was added in the above concentrations produced in our experiments vaso-constriction only. Similar experiments were made with pituitary extract (B. D. H.) and it was found that as much as ten times the amount necessary to give a slight constrictor effect to 30 c.c. of untraumatised blood would not prevent beginning vaso-dilatation when added to well-shaken blood, although with large concentrations of pituitary extract the vaso-dilatation was quickly cut short and followed by vaso-constriction (Fig. 5).

Fig. 5, Exp. 15. *a*. Shaken arterial blood plus 1 to 100 million adrenaline. *b*. Shaken arterial blood plus 1 to 1200 B. D. H. pituitary extract.

Still larger amounts of both adrenaline and pituitary extract completely prevented the vaso-dilator action of shaken blood and caused vaso-constriction from the beginning. It became apparent that the concentrations of adrenaline and pituitary extract which are necessary to

prevent the vaso-dilator effect created by traumatism are much higher than those to be expected in the arterial blood; consequently it is highly improbable that the vaso-dilatation is the result of the destruction of these substances.

The theory that the vaso-dilator action of traumatised blood is due to the formation or liberation of a vaso-dilator substance would seem the more plausible one. Injury to the red blood cells with consequent hæmolysis and liberation of vaso-dilator substances of a histamine-like nature was considered. In order to test this theory experiments were performed with different amounts of (*a*) blood traumatised in varying degrees, (*b*) laked blood, and (*c*) blood to which different amounts of histamine were added.

(A) *Traumatised blood.* The amount of traumatism was increased by shaking for increasing lengths of time either with or without glass beads. The quantity of blood circulated was varied by the use of flasks holding 30, 75, 220 and 475 c.c. respectively. When a 30 to 75 c.c. flask was used no extra blood was employed, the circulation being started through the flask filled with physiological salt solution. When a 220 or 475 c.c. flask was used it was filled at the start with heparinised blood from another dog and the circulation was allowed to pass through the flask until the two bloods were thoroughly mixed. No difference was noted in the effects of blood whether it was that of donor or recipient or a mixture of the two.

Fig. 6. Circulation of arterial blood shaken 20 minutes with bead.

When 30 c.c. of blood were used, it was found that a maximum dilator effect was obtained by shaking with a bead for approximately 2 minutes, and again as with blood shaken without a bead, there was no change in the degree of vaso-dilatation when the blood was shaken for 8 to 10 minutes. But shaking with a bead for 20 minutes produced

moderate vaso-dilatation followed quickly by limb constriction lasting for 3 or 4 minutes (Fig. 6). There is much froth formed by prolonged shaking so that 40 to 50 c.c. of blood were used in order to insure enough to fill the 30 c.c. flask. When 30 c.c. that had been shaken with a bead for 55 minutes were circulated immediate limb constriction and slight rise in limb pressure followed. Five minutes were required for the limb to return to its former volume, after which there was still further increase in volume and diminution in volume pulsations (Fig. 7).

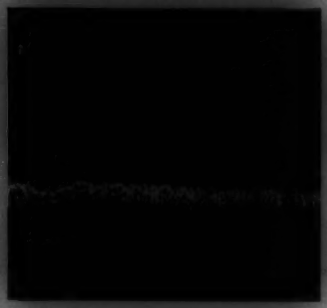

Fig. 7. Circulation of 30 c.c. blood shaken with beads for 55 minutes.

Increase in amount of blood circulated. A surprising finding was that when more than 30 c.c. of blood shaken for 2 to 5 minutes was circulated the vaso-dilatation did not increase proportionately with increase in the amount of blood. 75 c.c. produced slightly more effect than did 30 c.c. but 220 c.c. or 475 c.c. produced no greater nor more prolonged vaso-dilatation than did 75 c.c. Approximately 4 minutes were required for 475 c.c. to pass out of the flask; when that amount was circulated, vaso-dilatation reached its maximum in 25 to 35 seconds, and wore off in 60 to 90 seconds; limb volume and limb pressure then remained constant despite the fact that shaken blood circulated for about 2½ minutes more (Fig. 8). The vessels apparently acquire the property of resisting the vaso-dilator effect of such traumatised blood after approximately 30 seconds, and quickly assume their former contractile state, despite the fact that the same kind of blood still circulates in them. 475 c.c. of blood, shaken with beads in a Florence flask for 10 minutes caused vaso-dilatation about equal to that produced by blood shaken for 2 minutes, and no vaso-constriction followed. But

475 c.c. of blood that had been shaken with beads in 50 c.c. lots for 1 hour, so that the amount of traumatism was extreme, produced immediate diminution in limb volume and slight rise in limb pressure, similar

Fig. 8, Exp. 54. 1. Reactive hyperæmia. *a*. 30 c.c. arterial blood shaken 2 minutes. *b*. Circulated 475 c.c. arterial blood shaken with beads for 5 minutes. *c*. Approximate time when traumatised blood all out of flask.

to the effect of 30 c.c. shaken for 1 hour. The limb constriction lasted for 6 minutes after which it wore off and limb dilatation followed while limb pressure returned to normal. General blood-pressure remained unchanged throughout the experiment.

It was thus apparent that blood which had been slightly or moderately traumatised produced vaso-dilatation on circulation through the limb; but increase in the amount of blood circulated beyond 75 c.c. did not increase the amount or duration of the vaso-dilatation. The circulation of blood that was severely traumatised produced first vaso-dilatation followed by limb constriction and rise in limb pressure, and blood that was still more severely traumatised produced limb constriction and rise in limb pressure from the onset. Increase in the amount of severely traumatised blood circulated caused prolongation of the period of limb constriction.

To test for hæmolysis, equal quantities of blood that had been similarly shaken for varying lengths of time were centrifuged for 30 minutes and their plasma examined for the presence of hæmoglobin. The plasma of 50 c.c. of blood shaken by hand in a 100 c.c. flask up to 1 minute usually showed no hæmoglobin visible to the naked eye; but shaking for 2 to 3 minutes usually produced a definite reddish tint, and the greater the traumatism, the more intense became the

discoloration with hæmoglobin. Shaking for 1 hour produced very marked discoloration. The amount of hæmoglobin in each specimen of plasma was determined colorimetrically by comparison with whole blood, the hæmoglobin content of which was obtained by means of the Sahli hæmoglobinometer. In one experiment where whole blood contained 17·87 grm. of hæmoglobin per 100 c.c. the plasma of unshaken blood centrifuged for 30 minutes contained 0·0469 grm. per 100 c.c., that of blood shaken for 30 seconds 0·0573 grm. per 100 c.c., that of blood shaken for 1 minute 0·0677 grm. per 100 c.c., and that of blood shaken for 2 minutes 0·0791 grm. per 100 c.c. When blood was shaken with a bead for 12 minutes, the plasma contained 0·908 grm. per 100 c.c., when shaken for 20 minutes, it contained 1·418 grm. per 100 c.c., and when shaken for 55 minutes, it contained 3·689 grm. per 100 c.c. Thus unshaken blood showed after centrifuging only a faint hæmolysis, while after shaking for 55 minutes more than one-fifth of the hæmoglobin was liberated.

(B) *Laked blood.* In 16 experiments blood was laked by freezing and thawing. Freezing was accomplished most conveniently by immersion of the blood contained in Pyrex tubes in liquid oxygen. Estimations with the Sahli hæmoglobinometer showed about 90 p.c. of the hæmoglobin in the plasma. Amounts of the hæmolysed blood varying from 0·07 c.c. to 220 c.c. were circulated and untraumatised blood was diluted with different amounts of hæmolysed blood before circulation.

Injection of 0·07 c.c. of laked blood into the tube distal to the flask always produced a small amount of vaso-dilatation. The amount of vaso-dilatation increased with increase in the amount injected up to 0·5 or 0·7 c.c., dependent on the experiment and on the degree of hæmo-

Fig. 9, Exp. 80. *a*. Circulation of 30 c.c. of arterial blood shaken 2 minutes. *b*. 0·07 c.c. of 0·005 p.c. histamine. *c*. 0·07 c.c. of hæmolysed blood. *d*. 0·15 c.c. of hæmolysed blood. *e*. 0·7 c.c. of hæmolysed blood. *f*. 1 c.c. of hæmolysed blood.

lysis. Larger amounts gave no further increase and 1 c.c. usually produced at first some dilatation which was followed by slight constriction (Fig. 9). Larger amounts of hæmolysed blood were introduced into the untraumatised blood of the short circuited 30 c.c. flask by injection into the inlet tube and circulated. 3 to 5 c.c. produced marked limb constriction which was usually preceded by slight limb dilatation. 10 c.c. produced limb constriction only. 30 c.c. caused immediate and marked decrease in limb volume, increase in limb pressure of 3 to 7 mm. and rise in general blood-pressure, sometimes as much as 3 or 4 mm. (Fig. 10).

Fig. 10, Exp. 62. Circulated 30 c.c. hæmolysed blood.

Limb constriction lasted 3 to 7 minutes and was followed by limb dilatation and diminished volume pulsation lasting for an indefinite period.

In one experiment 220 c.c. of hæmolysed blood were circulated. Immediate and marked reduction in limb volume resulted which lasted for 9 minutes to be followed by moderate increase in volume and absence of volume pulsation. Both limb pressure and general pressure rose gradually during the period of 9 minutes, the former from 125 to 142 mm., and the latter from 130 mm. to 145 mm. The blood-pressure then gradually declined and returned to 130 mm. 10 minutes later.

Thirty c.c. of unshaken blood were diluted with various amounts of hæmolysed blood before circulation by withdrawal into a beaker containing the hæmolysed blood and immediate refilling of the flask with

the mixture. Vaso-dilatation was produced in increasing amounts when 0·2 to 1·5 c.c. of hæmolysed blood were used, but when 2 to 3 c.c. were used, vaso-dilatation was followed by slight limb constriction, and 5 to 10 c.c. usually produced limb constriction from the beginning lasting 2 to 4 minutes.

In one experiment varying amounts of hæmolysed blood were added to the 475 c.c. flask of arterial blood by injections into the proximal tube. One to three minutes were allowed for mixture which undoubtedly was incomplete. 2 c.c., 6 c.c., and 12 c.c. caused faint, brief limb dilatations when circulated, but no fall in general blood-pressure. 20 c.c. caused a temporary rise in limb pressure of 4 to 6 mm. of mercury and faint limb constriction. These findings are in marked contrast with those which follow where histamine was used.

(C) *Effect of addition of histamine.* Different amounts of blood were treated with varying amounts of histamine before circulation. In some of the experiments the blood was withdrawn into a beaker containing the histamine and returned immediately to the flask; in others, in order to exclude the effects of traumatism, the solution of histamine was injected in the proximal tube of the flask while it was short-circuited and then carried into the flask by directing the circulation through it for 1 to 2 seconds before short-circuiting it again; 2 to 3 minutes were allowed for mixture of blood and histamine.

In all experiments it was found that the greater the amount of histamine added to a fixed quantity of blood, the greater the vaso-dilator effects produced. When 30 c.c. of blood were used, 0·02 mgm. of histamine caused increase in limb volume and fall in limb pressure, about equal to that produced by blood that had been shaken for 2 minutes, without fall in general blood-pressure. 0·1 to 0·2 mgm. produced increase in limb volume and fall in limb pressure, followed in 30 to 40 seconds by fall in general blood-pressure of 10 to 20 mm. of mercury and further slight increase in limb volume. There was rapid recovery in 2 to 5 minutes. Larger quantities caused increasing fall in both limb and general pressure and prolongation of the period of recovery.

When histamine was added to 475 c.c. of blood it was found to produce vaso-dilatation, but enormously larger doses could be tolerated than when they were added to 30 c.c. of blood. Not only the blood vessels of the limb but also those of the general circulation rapidly acquired a remarkable power of resistance to continued vivi-perfusion with 475 c.c. of blood containing histamine. It required approximately 4 minutes for all the blood inserted into the 475 c.c. flask to pass out

of it. When, in an experiment on a dog weighing 10·5 kgm., 10 mgm. of histamine were added to 475 c.c. of blood, there resulted on circulation, a rapid increase in limb volume and fall in limb pressure followed by fall in general pressure (Fig. 11). But within 75 seconds these effects

Fig. 11, Exp. 64. 10·5 kgm. dog. Circulated 475 c.c. blood withdrawn and mixed with 10 mgm. histamine. Flask empty at ×.

had largely worn off despite the fact that blood containing the same proportion of histamine continued to enter the limb and from there the general circulation for at least 2½ minutes longer. On the other hand, when 30 c.c. of blood containing 10 mgm. of histamine were circulated, the blood-pressure in both femoral and carotid arteries fell rapidly to 15 mm. of mercury and the animal showed evidences of extreme and prolonged shock from which it had only partially recovered at the end of an hour.

Histamine was added in varying amounts to 30 c.c. of hæmolysed blood. It was found on circulation to counteract the constrictor effects and to produce vaso-dilator effects similar to but somewhat less marked than those produced when it was added to non-hæmolysed blood.

When the flask was connected with the femoral vein, the circulation of 475 c.c. of venous blood containing 2 mgm. of histamine caused a fall in general blood-pressure of 40 mm. Hg which was gradually recovered from in 10 minutes.

There was no instance of the reversed action of histamine as found by Dale and Richards when it was added to artificial perfusion fluids.

In four experiments 30 c.c. of blood were withdrawn and defibrinated before heparin was injected. On subsequent circulation, it was found to produce vaso-dilatation similar to that produced by shaken blood. Whipping for defibrination was kept up for 5 to 6 minutes. In two experiments, between 500 and 600 c.c. of blood from another dog were defibrinated by whipping for 15 to 20 minutes with a wire wisp. Circulation first of 30 c.c. and subsequently of 475 c.c. of this defibrinated blood produced vaso-constriction similar to that of larger quantities of hæmolysed bloods. The hæmoglobin content of the serum was not determined in any of the experiments with defibrinated blood but it was undoubtedly higher in the second set than in the first.

A comparison of the results obtained by circulation of varying quantities and intensities of traumatised and hæmolysed bloods reveals very great similarity of action. Blood traumatised to a slight or medium extent produces vaso-dilatation; when traumatised more extensively it produces vaso-dilatation followed by vaso-constriction, and when traumatised still more extensively it produces vaso-constriction only. Hæmolysed blood when injected into the tube in very small quantities or mixed with the unshaken blood of the 30 c.c. flask in somewhat larger quantities causes vaso-dilatation; when these quantities are moderately increased they cause vaso-dilatation followed by vaso-constriction and when they are markedly increased they cause vaso-constriction only.

Increase in the quantity of slightly or moderately shaken blood up to approximately 75 c.c. increases the intensity and duration of vaso-dilatation, but quantities greater than this produce no increase in the intensity or duration of the vaso-dilatation. The same is true of unshaken blood diluted with very small quantities of hæmolysed blood. Increase in the quantity of blood that has been traumatised to a point where it produces only vaso-constriction on circulation, causes prolongation of the period of vaso-constriction. This is also true of hæmolysed blood when circulated in moderate or high concentrations.

When the effects of traumatised and hæmolysed bloods are compared with those of blood treated with histamine, there are some points of similarity, and others of marked difference. There is a similarity of action in that circulation of 30 c.c. of untraumatised blood containing a very small amount of histamine causes vaso-dilatation in the limb without alteration of general blood-pressure; also further increase in the quantity of blood circulated containing histamine in this amount

does not proportionately prolong the period of vaso-dilatation. There is a marked difference in the action in that increase in the amount of histamine in a given quantity of blood beyond that which produces only dilatation in the limb, causes further limb dilatation and also dilatation of systemic capillaries with fall in general blood-pressure. The fall in general blood-pressure is also proportionately greater when the amount of histamine is increased, large amounts causing histamine shock.

The cause of the vaso-dilatation in the limb which results when mildly traumatised blood or a minute quantity of hæmolysed blood is circulated is apparently the same in either case and is a substance liberated from the broken down red blood cells.

The facts that increase in the traumatism or laking beyond a slight degree does not increase the vaso-dilator action, that marked increase causes vaso-constriction, and that the addition of increasingly greater amounts of histamine to blood whether untraumatised, traumatised, or hæmolysed always produces correspondingly greater vaso-dilatation, speak against histamine as the cause of the vaso-dilatation.

The cause of the vaso-constriction produced by the circulation of extremely traumatised or extensively hæmolysed blood may also be regarded as a substance liberated from the red blood cells, but active as a vaso-constrictor only when present in relatively high concentration.

The question of whether the vaso-dilatation produced by a small traumatism of blood and the vaso-constriction produced by an extensive traumatism are due to the liberation of different amounts of one and the same substance or of two different substances must at present be left open.

Summary and Conclusions.

1. The use of a vivi-perfusion apparatus devised by Dr G. V. Anrep is described.

2. Arterial or venous blood rendered incoagulable by heparin or hirudin acquires vaso-dilator properties when outside the body.

3. Traumatism of the blood up to a certain point hastens the development of vaso-dilator properties.

4. The vaso-dilator properties of such blood are independent of changes in O_2, CO_2, H-ion concentration, temperature, and exposure to air or light. They appear not to be the result of destruction of adrenaline or of pituitary principle while the blood is outside.

5. The plasma of such blood shows evidences of a slight amount of hæmolysis.

6. Laked blood, when injected in small quantities or circulated in high dilution with untraumatised blood also produces vaso-dilatation.

7. Blood that has been severely traumatised acquires vaso-constrictor properties. Its plasma shows a moderate or high degree of hæmolysis.

8. Moderate or high concentrations of laked blood also produce vaso-constriction when circulated in larger quantities.

9. These vaso-motor changes are apparently due to the action of the hæmolysed blood which when present in the circulated blood in small amounts causes vaso-dilatation and in large amounts causes vaso-constriction.

10. The vaso-dilator properties of traumatised blood are as far as the evidence shows not due to the liberation of histamine.

11. Untraumatised blood collected from the femoral vein during the period of reactive hyperæmia following arterial obstruction possesses no special vaso-dilator properties.

12. Blood vessels when perfused with large quantities of blood possessing vaso-dilator properties, whether due to traumatism or adding hæmolysed blood or histamine, dilate at the onset but rapidly acquire the power of resisting the vaso-dilator action.

Thanks are due to Dr Anrep for assistance throughout the course of the work, and Prof. Lovatt Evans for suggestions in connection with the work on hæmolysed blood.

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THE NERVOUS MOTIVE ENERGY.

REPLY TO SYBIL COOPER AND E. D. ADRIAN.

By I. ATHANASIU (*University of Bucarest*).

Since Piper's(1) demonstration, by means of the string galvanometer, that the action current of the muscles in voluntary contraction shows a great number of oscillations during the unit of time, the question arose whether the rhythm of these oscillations was derived from the muscles or the nervous system.

The controversy which arose over this question between Garten(2) who attributed it to the muscle, and Piper(3) who attributed it to the nervous system, is already known.

Forbes and Rappleye(4), in trying to elucidate the problem by experiments made on man, warmed and cooled the muscle of the fore-arm to which were applied non-polarisable electrodes. They found that the frequency of the oscillation in the electromyogram rises with the warming and diminishes with the cooling of the muscle, which should prove that their rhythm depended on the muscle and not on the nervous system. The latter is supposed to send to the muscle, according to these authors, 300–1000 vibrations per second and even more. But they do not bring any experimental proofs to back their views.

Cooper and Adrian(5) have taken up the question and made experiments on frogs with brains destroyed. Their researches aimed at distinguishing the share of the muscle from that of the nervous system in the rhythm of the oscillations in the reflex electromyogram and to that end they separately and alternately cooled the muscle and the spinal cord. When the muscle was cooled, the cord was kept by certain methods at a constant temperature, and vice versa. The non-polarisable electrodes were placed on the gastrocnemius muscle and a fore-limb was stimulated either by mechanical or electrical means (induction shocks). The photographic registering apparatus moved at a speed of 15 cm. per sec. The authors do not mention the physical constants of the string of the galvanometer.

Cooper and Adrian have counted, in the reflex electromyogram thus obtained, only the large oscillations on one side and on the other the

total number. They found that cooling the muscle (the temperature of the spinal cord remaining constant) causes little change in the rhythm of oscillation in the reflex electromyogram, but that cooling the cord (the temperature of the muscle remaining constant) changes it more.

Thus taking the average of the number given by these authors, we find:

(*A*) *Cooling the muscle.*

Temperature	Total number of oscillations per sec.
11°–11·5°	118
20°	122
22°–25°	128

(*B*) *Cooling the cord.*

5°	95
6°	105
7°	118
14°	126
20°	145

From these experiments Cooper and Adrian concluded that the rhythm of the oscillations in the reflex electromyogram follows exactly the frequency of nervous discharge of the spinal cord, which would be about 120–150 per sec. in the frog at room temperature. Consequently the rhythm depends on the nervous system and not on the muscle. It is supposed that the number of nervous vibrations is not superior to the frequency of the muscle response. The authors state at the same time that when warming the cord the large oscillations become more numerous and their intervals more regular. In order to explain this phenomenon, they suppose that the amplitude of these oscillations depends on the number of muscle fibres contracted at the time and that this number would be higher when the cord is warmed, because of a larger number of the motor neurones coming into action for the same movement.

This summarises as faithfully as possible the results reached by Cooper and Adrian.

Commenting on these results, they find them in contradiction with those I have obtained in my own work on the nervous motive energy (6).

We shall separately examine each question discussed by these authors.

1. *The existence of electro-neuro-motive oscillations in the voluntary and reflex electromyogram.* My researches allowed me to distinguish in every electromyogram, voluntary or reflex, the presence of two kinds of oscillations: some of variable amplitude (large and middle-sized) which

I have called *electro-muscular* and the others of very small but regular amplitude, appearing in groups of 2-5, called *electro-neuro-motive*.

In order to be able properly to distinguish these two kinds of oscillations, we may take the following records: Figs. 1 and 2, Plate I, showing voluntary electromyograms of the flexor muscles of human fingers and Figs. 4 and 5, Plate II, showing reflex electromyograms of the gastrocnemius of the frog. The groups of small oscillations included between the white dotted lines are those which I call electro-neuro-motive; all the others are the electro-muscular ones. The rhythm of the first ones is marked above each group. I will briefly point out the reasons which lead me to consider that those small oscillations represent the action current which accompanies the nervous motive energy.

Fig. 1.

Fig. 2.

Fig. 1. E = Direct electromyogram of a neuromuscular preparation. Number of electric stimuli applied to the nerve = 982. M = Myogram. T = Time in one-hundredths of a second. String of silvered glass. Diameter = 2·5μ. Resistance = 6500 ohms. Tension = 1·5 mm. per 0·001 v.

Fig. 2. Reflex electromyogram of frog, without brain. Exterior temperature = 24°. Gastrocnemius muscle. String of silvered glass. Diameter = 2μ. Resistance = 9000 ohms. Tension = 0·8 mm. per 0·001 v. An amplifier in the circuit. Mechanical stimulation of the fore-limb. T = Time in one-hundredths of a second.

When we register the action current of a muscle-nerve preparation, after the nerve has been stimulated, one can see that up to 300 stimuli per sec. the oscillations of the galvanometer follow the frequency of the stimulus very exactly, though their amplitude lessens on account of the fatigue. Above 300 stimuli per sec. the form of the electromyogram becomes more and more irregular and approaches the reflex or voluntary electromyogram. Fig. 1 is a direct electromyogram and Fig. 2 a reflex electromyogram of the frog.

The likeness between the two is perfect; the same large, middle-sized and small oscillations are in the two. The small oscillations, of regular amplitude, which are in the direct electromyogram, follow between certain limits the rhythm of the stimulus. What other conclusion can be drawn from this likeness, unless that the muscle in voluntary or reflex contraction also receives a number of stimulations greater than 300 per sec.? And if we consider the groups of quite small oscillations as representing the nervous stimulus, we find in fact that their frequency is included between 300 and 550 oscillations per sec.; in the hot summer time we have even found 600 per sec.

The very irregular form of the other oscillations (large and middle-sized) which represent the action currents of the muscular contraction, may be explained by the intervention of the refractory period of the muscle, which puts a limit to the frequency of its responses. The following diagram (Fig. 3) shows the mechanism by which this refractory period has such an effect on the form of the electromyogram (voluntary or direct).

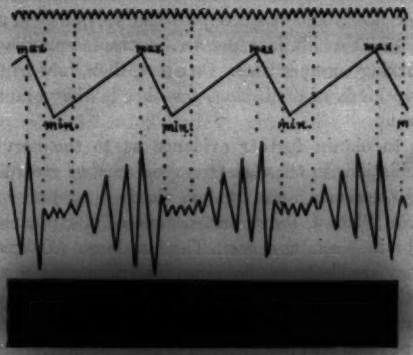

Fig. 3.

Fig. 4.

We suppose a rhythmic stimulus giving a certain number of interruptions in the unit of time. The muscle having phases of maximum and minimum excitability, the intensity of its responses—expressed by the amplitude of the electromuscular oscillations—will be strongest during the maximum phase and weak during the minimum phase. It is even possible that the muscle may not respond at all during this last period, and if so the galvanometer will record only the action current which accompanies the nervous motive energy. This action current is expressed by the small oscillations of a regular amplitude, which are to be seen as well in the direct electromyogram as in the voluntary and reflex one.

One may get reflex electromyograms from cooled frogs the forms of which considerably resemble our diagram, as may be seen in Fig. 4. The large oscillations are sufficiently regular. There are among them middle-sized and groups of quite small ones. Our explanation therefore finds a very important support in this electromyogram. Again Cooper and Adrian show in Fig. 6 of their paper an electromyogram of the frog with the cord cooled to 4·5°, which bears a very close likeness to our diagram.

We can conclude from the preceding observations that the oscillations of every electromyogram, reflex or voluntary, have two different origins: (*a*) the large and middle-sized are muscular waves as far as the energy is concerned, and show the action current which accompanies the muscle twitch; (*b*) the very small ones are of nervous origin, and represent the action current which accompanies the nervous motive energy.

The frequency of the first is generally between 80 and 150 per sec. in the mammalia and frog, reaching even 200 in the latter during the great summer heat. The frequency of the second is much higher; it comprises 300–500 oscillations per sec. in the mammalia and 300–600 in the frog during summer time. One sees, then, how irrational it is to add these two kinds of oscillations together in the voluntary or reflex electromyogram.

In order to obtain further evidence as to the nervous origin of the small oscillations in those electromyograms we recorded the action current of the nervous motor system (brain + cord or brain + sciatic nerve). Fig. 3, Plate I, shows an example of electroneurogram of the guinea-pig (brain + sciatic nerve). The two large oscillations belong to the heart; they are electrocardiograms. The numerous small oscillations are sufficiently alike to those of the voluntary electromyogram as to their form and number.

But Cooper and Adrian mention in their memoir: "Our own records

of the normal electromyogram (of the frog, the cat and man) show occasional clusters of very small waves of high frequency, but we have never been able to make out any clear separation of two such types of waves as Athanasiu describes, since there are always many of intermediate size. It should be added, however, that we have not made any detailed statistical examination of our records." These statistics have been made by me upon hundreds of records and I consider them as accurate.

If these authors did not succeed in clearly separating the two kinds of oscillations described above, the explanation of their failure is quite simple. It consists in the slow speed of their registering apparatus, which did not exceed 150 mm. per sec., whereas I insist in my work upon the fact that 500 mm. per sec. is necessary at least.

The number of electro-neuro-motive oscillations being of 300–600 per sec. in the frog in summer, it follows that one millimetre of the record of these authors must contain 2–4 of the small oscillations, a condition materially impossible, because the shadow of the string itself only measures 3–4 tenths of a millimetre. The frequent black spots seen on those records are due to nothing else than the electro-neuro-motive oscillations heaped together.

Moreover, we do not find in the work of these authors the physical constants of the string used and especially its tension. I have demonstrated already that a string under slight tension cannot follow the rhythm of rapid oscillations, and if such has been the case with Cooper and Adrian's experiments it is still easier understood why they have not been able to observe the electro-neuro-motive oscillations properly. My researches have shown that Piper's fear of tightening the string, on account of its own vibrations which would interfere with the record, was by no means justified. On the contrary, the tight string follows with the greatest fidelity the swiftest variations of the current, without any interference, as I have convinced myself by repeatedly testing the string galvanometer.

Therefore Cooper and Adrian's objection, on the existence and the meaning of the electro-neuro-motive oscillations, is by no means justified.

(2) *The large and middle-sized oscillations represent the action current of the muscle twitch.* In my researches on the action current of the muscle in voluntary contraction, I showed that the large and middle-sized oscillations of the electromyogram are the expression of the action current of the muscle twitch. The following additional proofs may be brought in support of this.

(a) When we record the action current of a muscle-nerve preparation (gastrocnemius muscle + sciatic nerve) successively in isotonic and isometric contraction, we find that the amplitude of the oscillations is considerably diminished in the second case, because the muscle is unable to contract and gives a very faint twitch. In Figs. 5 and 6 this is shown very clearly.

Fig. 5.

Fig. 6.

Fig. 5. E = Direct electromyogram of a neuromuscular preparation. Frequency of stimulation applied to the nerve = 569 per sec. Isotonic contraction. M = Myogram. String of silvered glass. Diameter = 2·5μ. Resistance = 6500 ohms. Tension = 1·5 mm. per 0·001 v. Time in one-hundredths of a second.

Fig. 6. E = Electromyogram of the same preparation in isometric contraction. M = Myogram. Same string and same tension.

(b) By the help of a special apparatus, described in my first work (p. ii) I have, in collaboration with Bull, recorded the longitudinal vibrations of the flexor muscle of the fingers and their action current, with two different string galvanometers, as it is shown in Fig. 7.

We obtained the records I and II. The first one (I) is the voluntary electromyogram of the flexor muscles of the fingers; II represents the longitudinal vibrations of the same muscles (muscle twitch), converted into electric vibrations by the apparatus F held in the hand. If the

contraction of the muscle were perfectly continuous the record II would be a straight line, because no current could have been produced in the

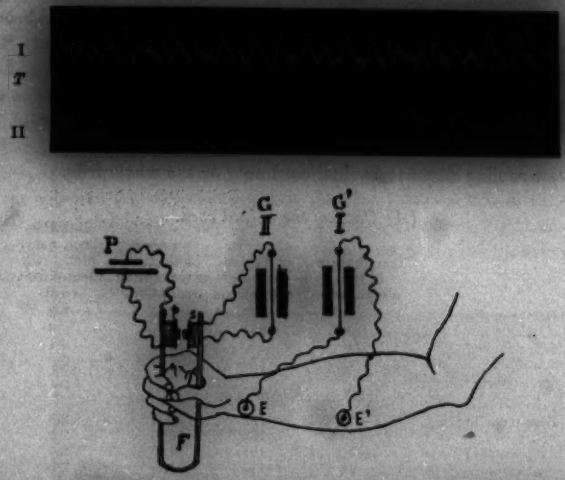

Fig. 7. I = Voluntary electromyogram of the flexor muscles of the fingers given by the galvanometer *G'*. *T* = Time in one-fifth of a second. II = Electric record of the longitudinal vibrations of the same muscle, given by the galvanometer *G* and the apparatus *F* held in the hand. The first coil receives the current from the battery *P*. The secondary coil (*s*) is in the circuit of the galvanometer *G*. *E* and *E'* = Non-polarisable electrodes. *I* = String of silvered glass. Diameter = 3·5μ. Resistance = 6500 ohms. Tension = 1·5 mm. per 0·001 v. *II* = String of silvered glass. Diameter = 3μ. Resistance = 7000 ohms. Same tension as *I* string.

secondary coil (*s*). But we see that this record has on the contrary a great number of oscillations, which almost copy the form of the large and middle-sized waves of the electromyogram. Consequently both have the same origin and represent the twitches of the muscle when in continuous contraction (tetanus). That is the reason why I have called the large and middle-sized oscillations of the voluntary or reflex electromyogram electromuscular oscillations. They are of muscular origin, as far as the energy is concerned, because they represent the action current which accompanies the muscle twitches. But as to their frequency and amplitude they depend directly on the nervous system, just as the oscillations of the direct electromyogram depend on the stimulus applied to the

nerve. Cooper and Adrian are therefore unjustly attributing to us the opinion, similar to Garten's, that the rhythm of these oscillations is due to a peculiar quality of the muscle.

If these authors had been able to record distinctly the two kinds of oscillations (the electromuscular and electro-neuro-motive ones) they would have seen that cooling the cord diminishes the frequency of both of them, as we have shown by repeating the experiments of Cooper and Adrian. We give in the following table the average number of the electromuscular and electro-neuro-motive oscillations in the frog, before and after cooling either the spinal cord only or the whole frog.

(*A*) *Cooling the spinal cord.*

	Temperature in the œsophagus	Number of electro-muscular oscillations	Number of electro-neuro-motive oscillations	Relation Elect.-neur.-mot. oscil. / Elect.-musc. oscil.
Before cooling	23 – 25°	170	545	3·2
„ „	23 – 25°	201	583	2·9
After cooling	7·8–10°	130	440	3·4
„ „	7·8–10°	135	412	3·0

(*B*) *Total cooling.*

Before cooling	21 – 24°	192	500	2·6
„ „	21 – 24°	176	516	2·9
After cooling	7 – 10·5°	165	430	2·7
„ „	7 – 10·5°	138	455	3·3

It follows from the above table that cooling only the cord diminishes the frequency of the electromuscular oscillations and therefore the frequency of the muscle twitches too, as has been demonstrated by Cooper and Adrian. But it diminishes also the number of electro-neuro-motive oscillations, therefore, the number of stimulations sent by the nerve centres to the muscle. The rhythm of the former is consequently strictly dependent on the rhythm of the latter, as I have affirmed, and all that affects the nervous centres necessarily influences the two sorts of oscillations. Such is the effect of poisoning these centres by alcohol(7), which—like cooling—diminishes the number of the neuro-motive vibrations, from which there results a diminution in the number of muscle twitches.

Figs. 3 and 4 (Plate II) are examples of reflex electromyograms of gastrocnemii muscles of the frog cooled either entirely or the spinal cord only.

(3) *The frequency of the nervous-motive vibrations is superior to the number of responses that the muscle can give.* We quote Cooper and Adrian's memoir: "Cooling the cord should reduce the frequency of the

impulses in the nerve, but if this is still too rapid for the muscle to follow, the effect would be either to leave the muscular response unchanged or else to make its frequency increase." Our studies of the direct electromyogram showed us that its form becomes similar to that of the voluntary electromyogram as soon as the frequency of stimulation applied to the nerve surpasses the frequency of response which the muscle can follow. Now the mere irregularity of the voluntary electromyogram suffices to indicate that the number of nervous impulses must surpass the limit of the muscle response, because, if the number were within these limits, as Cooper and Adrian suppose, the amplitude of the oscillations of the voluntary electromyogram would be regular, as it is in the direct electromyogram with stimulation not above 300 per sec. Fig. 8 shows, in fact, the form of a direct electromyogram with the nerve stimulated with 157 excitations per sec.

Fig. 8. *E* = Direct electromyogram of gastrocnemius muscle of the frog. Number of stimulations applied to the nerve = 157 per sec. *M* = Myogram. *T* = Time in one-hundredths of a second. String of silvered glass. Diameter = 2·5μ. Resistance = 6500 ohms. Tension = 1·5 mm. per 0·001 v.

Such would be the form of the reflex electromyogram if the opinion of Cooper and Adrian were correct, namely: "that the maximum rate of discharge from the cord in the frog at room temperature is 120–150 per sec., and that the frequency of the oscillations in the electromyogram is usually identical with the frequency of discharge from the cord." Now, on the contrary, the form is very irregular; but this does not happen by chance. There is a close enough relation between the number of nervous motive vibrations and that of the muscular responses included in every voluntary or reflex contraction. In fact I have found in records of

voluntary electromyograms made in a series of animals(8) that this relation is almost a constant quantity. The relation oscillates around the number 4, whether it is caused by a very quick movement, as the flight of a bird, or a very slow one, as the progress of the snail. Thus the average is four nervous vibrations for one muscle response (twitch); and if the nervous centres are cooled or poisoned by alcohol and thus diminish the frequency of the vibrations sent to the muscle, one distinguishes at the same time a proportional reduction in the frequency of the muscle response notwithstanding the fact that the muscle still receives a number of nervous stimulations superior to its rate of response. These experiments prove that one cannot identify the experimental tetanus with the voluntary or reflex one. Consequently Cooper and Adrian's objections are not well founded.

(4) *The functional synchronism of the nervous and muscular elements.* In order to explain the differences of amplitude in the voluntary and reflex electromyogram Cooper and Adrian produce the following hypothesis: "The explanation we take to be that the large waves represent the simultaneous discharge of impulses from the majority of the nerve cells and consequent contraction of the majority of the muscle fibres and that the small waves represent the discharge from a small number of nerve cells out of phase with the rest." It is therefore the theory which Gotch(9) put forward for the nerves and which these authors apply to the nervous centres. But if the neurones can act individually or associate into a larger number when commanding a higher effort, it is not the same for the muscle fibres. I have explained in my second memoir (pp. 21, 22) why the "all or nothing" rule cannot be applied to the muscle of the skeleton and why the muscle fibres cannot act separately or by small groups as Keith Lucas(10) and Pratt(11) have maintained. One of the principal reasons against that conception is the structure of the muscle. Working with Dragoiu I have proved(12) the existence of a very abundant and very tight connective tissue between the fibres of the skeleton muscle by the aid of impregnation with reduced silver nitrate, according to Cajal's method.

One can clearly see in these preparations both the envelope of every fibre, or sarcolemma which is of connective elastic nature, and the interstitial connective tissue, which binds the muscular fibres together very tightly. In face of the fact that such is the structure of the striated muscle, Pratt's opinion, that the fibres of the muscle can freely slip over one another, becomes a material impossibility. And Pratt relies in his experiments just on this slipping of the fibres, in order to prove that the

contraction of a single fibre in the muscle must be considered as reaching its maximum and that consequently the fibre obeys the law of "all or nothing." Such a slipping cannot be admitted for the muscular fasciculus either, and in that case the experiments of Keith Lucas are equally inconclusive in proving the fitness of that law to the muscle of the skeleton. In fact, what would happen if a single fasciculus were contracted? The force developed in it would be transmitted, by the corresponding tendinous fibres, directly to the resistance to be overcome, *i.e.* to the part of the bone which should be moved. To this resistance is added that of the whole mass of the muscle which is at rest. As it cannot overcome the whole of these resistances, it must result that all the chemical energy employed by the muscular fasciculus for its supposed contraction will be transformed into heat, since there is no mechanical work. Even if we suppose that the number of fasciculi contracted at the time were sufficiently great to move the corresponding bony lever, there would still be an unnecessary quantity of energy wasted in moving the great mass of the muscle which is at rest.

For all these reasons, of structural and functional nature, I submit that all the fibres of the muscle contract at the same time when performing a certain work. The value of the work depends on the quantity of energy spent by each of these fibres and not on their number. Thus the functional synchronism is an essential condition of the muscular work.

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Note. The Editors deeply regret the death of Prof. Athanasiu which prevented him from seeing this paper in proof. The manuscript (undated) was found after his death by Prof. Marinesco of Bucarest and has been kindly seen through the press by him.

DESCRIPTION OF PLATES.

Plate I.

Figs. 1 and 2. Voluntary electromyogram of the flexors of the fingers in man. String of silvered glass. Diameter = 3μ. Resistance = 4000 ohms. Tension = 2 mm. per 0·001 v. Magnification = 2000 times. Time in fifths of a second.

Fig. 3. Guinea-pig. Electroneurogram. Brain and sciatic nerve. Voluntary walk. String of silvered glass. Diameter = 2·5μ. Resistance = 6500 ohms. Tension = 2 mm. per 0·001 v. Magnification = 1000 times. Time in fifths of a second.

Plate II.

Figs. 1 and 2. Frog with brain destroyed. Reflex electromyogram of gastrocnemius muscle. Temperature on the outside 27°. String of silvered glass. Diameter = 2μ. Resistance = 9000 ohms. Tension = 0·8 mm. per 0·001 v. Amplifier in the circuit. Mechanical stimulation of fore-limb. Time in one-hundredths of a second.

Fig. 3. Frog with brain destroyed. Total cooling. Temperature in the œsophagus, 8·7°. Reflex electromyogram of gastrocnemius muscle. Same string as in Figs. 1 and 2. Mechanical stimulation of fore-limb. Time in one-hundredths of a second.

Fig. 4. Frog with brain destroyed. Cooling of spinal cord. Temperature in the œsophagus = 9·5°. Reflex electromyogram of gastrocnemius muscle. Mechanical stimulation of fore-limb. Same string as in Figs. 1 and 2. Time in one-hundredths of a second.

Fig. 1.

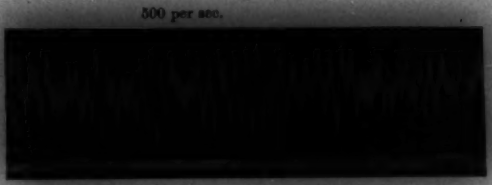


Fig. 2.

Fig. 3.

500 per sec.

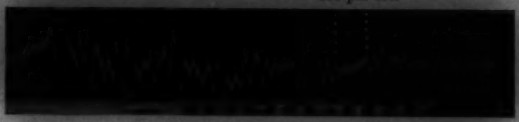
Fig. 1.

500 per sec.

Fig. 2.

460 per sec.

Fig. 3.



375 per sec.

Fig. 4.

COMPARATIVE EFFECT OF VARIOUS DRUGS UPON THE CORONARY CIRCULATION.

By G. V. ANREP and R. S. STACEY (*Scholar of Trinity College, Cambridge*).

(*From the Physiological Laboratory, Cambridge.*)

In a series of recent papers(1, 2, 3) a method has been developed for registering the outflow of blood from the drained coronary sinus during a single cardiac cycle by means of the hot-wire anemometer. The records already obtained by this method show three characteristic waves of increase in outflow and the relations of these waves to other events of the cardiac cycle have been worked out. The first wave is caused by and follows the contraction of the auricle; the second and smallest is related to the isometric period of ventricular systole, while the third and largest wave is determined by the contraction of the heart during the ejection phase. It had further been noticed that these three waves, which occur under widely different conditions of the heart beat, may have a very different form. In some experiments they are sharply defined, in others they merge one into another up to a point of complete fusion. When one or the other type of the coronary outflow was observed it would generally persist during an experiment, except in prolonged experiments in which the separate waves show a tendency to fuse together. The difference between the various types of the coronary outflow is independent of changes in such circulatory conditions as arterial blood-pressure, cardiac output and heart rate.

The question remains as to whether the differences in the coronary outflow curves depend on the strength of the cardiac contraction and it was the object of the experiments described below to obtain information on this point. The changes in the strength of contraction were produced by administration of certain drugs whose effect upon the heart and upon the coronary circulation is already known.

The experiments were performed on dogs, using the heart-lung preparation. The coronary blood flow was recorded by the method described by Anrep, Cruickshank, Downing and Subba Rau(1). The heart rate was controlled in all experiments by stimulating the right auricle.

The drugs used were adrenaline, carbon dioxide, pituitary extract and caffeine. These drugs have a definite effect upon the coronary circulation as well as upon the heart muscle. It is, therefore, impossible, without further analysis to decide whether a change in the general appearance of the coronary outflow curve which follows the administration of any of these drugs is due to a decrease or increase in the coronary blood flow or to changes in the contraction of the heart. In order to distinguish between these factors we examined the effect of the drugs, first allowing them to exert their full effect upon the heart and upon the coronary blood flow, and then under conditions in which the coronary blood flow was kept constant by adjusting the aortic blood-pressure. We could thus compare the effect of the drugs at constant aortic pressures and at constant coronary blood flows, as will be made clear from the description below.

The effect of adrenaline and carbon dioxide. Adrenaline diminishes the volume of the heart and increases the strength of its contraction, carbon dioxide acts in the opposite manner; both, however, increase the coronary circulation. After a number of records of the normal coronary outflow had been obtained adrenaline or carbon dioxide was administered and a second set of records was taken. The latter records showed the effect of the drug on the calibre of the coronary vessels themselves, they were therefore not suitable for comparison with the normal records. In order to allow a more direct comparison the aortic blood-pressure was lowered to a point at which the coronary blood flow became equal to that observed before the administration of the drug. A third set of records was then taken.

In some experiments the effect of the drug was allowed to wear off, after which we increased the blood-pressure up to a point at which the coronary blood flow became equal to that observed during the effect of the drug, a fourth set of records being taken.

Fig. 1 shows three redrawn superimposed curves taken from a typical experiment: a normal curve, a curve after administration of adrenaline and a third curve after administration of carbon dioxide. The three curves are taken at a constant mean aortic blood-pressure. Fig. 2 shows similar curves but after adjustment of the blood-pressure to a level at which the coronary blood flow was constant in the three cases.

In this experiment adrenaline and carbon dioxide increased the coronary circulation to nearly the same extent (Fig. 1); the individual waves of the coronary outflow became more distinct after adrenaline, while during administration of carbon dioxide they flattened out and

fused with one another. This change in form of the curves persisted after adjustment of the aortic pressure (Fig. 2). The position of the

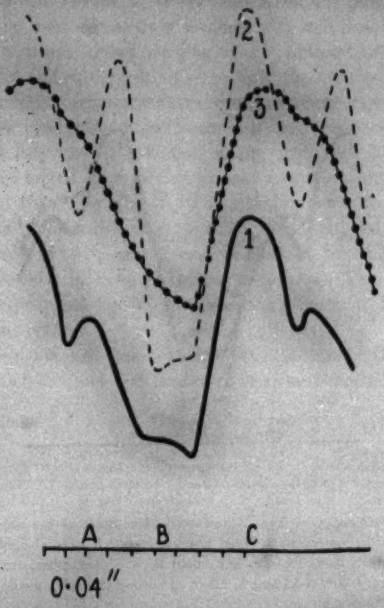


Fig. 1. Redrawn, superimposed and corrected curves of coronary outflow recorded by the hot-wire anemometer. Mean arterial blood-pressure 95 mm. Hg; systemic output is maintained at 650 c.c. and the heart rate at 150 beats per minute: 1, normal curve, coronary blood flow—0·45 c.c. per beat; 2, effect of adrenaline (0·01 mgm.)—0·73 c.c.; 3, effect of carbon dioxide (7·3 p.c.)—0·65 c.c.; *A*, first or auricular wave; *B*, second wave, related to the isometric period of ventricular contraction; *C*, third wave occurring during the ejection period.

waves in the cycle was not appreciably altered except that the interval between the auricular wave and the first ventricular wave was shortened after adrenaline and lengthened after carbon dioxide; a change which is probably determined by the effect of these drugs upon the A.–V. conduction[1]. It is known that the strength of the heart beat is affected by

[1] The position of the waves in the cycle was determined in experiments in which the coronary outflow was registered together with the intra-ventricular and aortic pressure.

considerably smaller concentrations of adrenaline or carbon dioxide than is the coronary blood flow. Administration of either of the drugs in

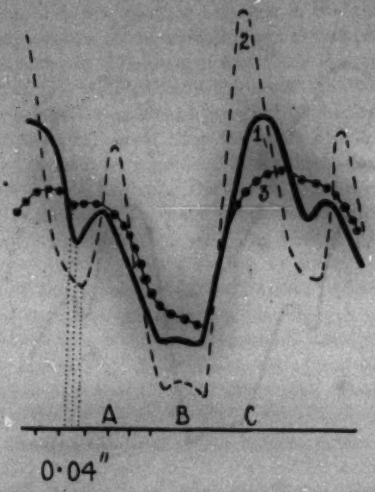


Fig. 2. Same experiment as in Fig. 1, but after adjustment of the aortic blood-pressure so as to maintain the coronary blood flow constant: 1, normal curve, aortic pressure 95 mm., coronary flow 0·45 c.c. per beat; 2, effect of adrenaline, aortic pressure 78 mm., coronary flow 0·45 c.c.; 3, effect of carbon dioxide, aortic pressure 82 mm., coronary flow 0·46 c.c.

such small concentrations has a similar but less conspicuous effect upon the form of the coronary outflow curve to administration of the higher concentrations. In these cases an adjustment of the aortic pressure is not, however, required since the amount of the coronary blood flow remains unaffected by the drug.

The effect of pituitary extract and caffeine. Pituitary extract and caffeine in the doses which were used had only a slight effect upon the heart muscle as measured by its diastolic volume. Pituitary extract, however, produced a conspicuous diminution and caffeine an increase in the coronary circulation. After readjusting the blood-pressure so as to maintain the coronary circulation constant we found the curves to be nearly superimposable on the normal curves except that after the

pituitary extract the rate of rise of the main wave became somewhat slower while after caffeine it became steeper.

Summarising the results of these experiments it can be said that with none of the four drugs used is there any relation between their effects upon the heart muscle and upon the amount of the coronary blood flow. The following table shows that the changes in the heart volume (which at a constant heart rate, resistance and output is a measure of the strength of contraction(4)) and the changes in the coronary blood flow are independent of one another. The data given in the table are taken from an experiment in which the drugs were administered in the order shown in the table; the blood used in the preparation was replaced by fresh blood, after administration of adrenaline and pituitary extract, and time was allowed for the heart volume and the coronary blood flow to return to normal. The weight of the heart in this experiment was 62 grm. and the amount of blood in circulation was about 650 c.c.

The total output and heart rate were maintained throughout the experiment at 800 c.c. and 156 beats per minute respectively.

	Change in coronary blood flow in p.c.	Change in heart volume before and after adjustment of the blood-pressure, in c.c.		Change in mean aortic blood-pressure required for adjustment, in mm. Hg
		Before	After	
Adrenaline 0·01 mg.	+ 85	−8·4	−9·5	From 100 to 80
Carbon dioxide 7 p.c.	+ 70	+7·5	+4·3	„ 100 „ 87
Pituitary extract 0·1 c.c. (B.D.H.)	− 75	+2·4	+7·8	„ 100 „ 145
Caffeine sodium benzoate 0·1 grm.	+115	−1·6	−4·5	„ 100 „ 55

Although there is no relation between the effect of these drugs upon (*a*) the amount of coronary blood flow and (*b*) the strength of contraction, the latter determines the character of the outflow curves. When the heart beat is strengthened by administration of adrenaline the waves of the coronary outflow are sharply defined, whereas when it is weakened by carbon dioxide the separate waves fuse together. The effect of pituitary extract and caffeine in the doses used is in this respect negligible. But larger doses of pituitary extract have an effect similar to that of carbon dioxide, while large doses of caffeine produce changes similar to adrenaline.

SUMMARY.

From the study of the effects on the heart of adrenaline, carbon dioxide, pituitary extract and caffeine it is concluded that the effect of these drugs upon the strength of heart beat as measured by the heart volume bears no relation to their effect upon the amount of coronary blood flow. But the form of the coronary outflow curves is dependent on the strength of the cardiac contraction, the three characteristic waves being accentuated during the increased strength of contraction produced by adrenaline and obliterated during the weakened contraction as a result of administration of carbon dioxide. Small doses of pituitary extract and caffeine produce a negligible effect on both the strength of contraction and the form of the coronary outflow curve.

The expenses of this research were defrayed from a grant by the Medical Research Council held by one of us (G. V. A.).

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IS THERE "TRANSITIONAL DECREMENT" IN NARCOTISED NERVE?

By G. KATO and D. TERUUCHI.

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In 1923 one of the present writers(1) made the first report on the decrementless conduction in a narcotised region of nerve. Two kinds of results were obtained on this problem by experiments in a narcotising chamber. They are as follow:

A. The time required for suspension of conduction from an outside electrode is not dependent upon the length of narcotised nerve, if the narcotised region is longer than 6 mm.

B. On the other hand, the time is dependent upon the length of narcotised nerve, if the narcotised region is shorter than 6 mm. ("limit-length"); in other words, below the "limit-length," the shorter the narcotised region, the longer time is required to suspend conduction.

These two facts were demonstrated at the XIIth International Physiological Congress at Stockholm.

The result A indicates that there is non-decremental conduction in a narcotised region of nerve under the stated condition. And as to the result B we offered the following explanation: The whole nerve within the narcotising chamber is in the same condition, and therefore, theoretically speaking, the depth of narcosis ought to be uniform over the entire length of nerve within the chamber. But this is not the case in reality as it will be seen in Fig. 1. The parts of nerve near the edge of the narcotising chamber receive the influence from the normal part outside, for instance by the diffusion of the narcotic out of the chamber along the fibres and by the inward diffusion of the normal tissue fluid or of Ringer's solution along the fibres from outside, giving a gradient of concentration of narcotic so that full depth of narcosis is only reached at a distance of about 3 mm. from the chamber wall. The gradient of narcosis thus made may be represented diagrammatically by Fig. 1. The Fig. 2 shows the case in which the "limit-length" is narcotised. If, therefore, the stretch of nerve to be narcotised is sufficiently short, no part of the nerve within the narcotising chamber can be free from the influence from the normal parts above and below as it is shown in

Fig. 3, and the narcosis would proceed only to the point P at the stage of narcosis at which it would have proceeded to F in a longer region.

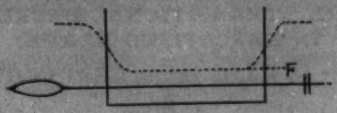
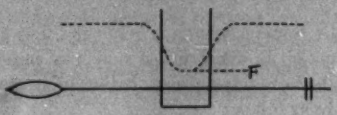


Fig. 1.



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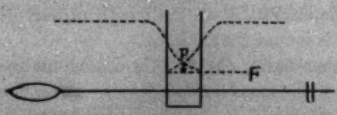

Fig. 3.

Thus the narcosis proceeds always slower in such a short region than in a longer one. If we assume that the depth F of narcosis is needed for abolition of conduction from the outside electrode, then a longer time is required for it in a shorter region, because the narcosis has to proceed from P to F. The "limit-length" is about 6 mm. in the case of 2·0–3·0 urethane or cocaine solution.

This point has, two years later, received an experimental proof by T. Hayashi(2) who has determined the excitability of nerve within and without the narcotising chamber by means of a sharply localised mechanical stimulus.

Previous to Hayashi's experiment, A. Forbes and his collaborators(3), who also have proved the non-decremental conduction in a narcotised region with mammalian nerve, suggested the alternative possibility of "transitional decrement," saying that on passing from normal to narcotised nerve the impulse does not fall immediately to the lower level characteristic of narcosis, but is conducted with a decrement for a short distance before reaching its new level, although the local response at every individual point on the narcotised nerve is strictly all or none. This suggestion seems apparently to agree with the results of Drury(4) and of Cattell and Edwards(5).

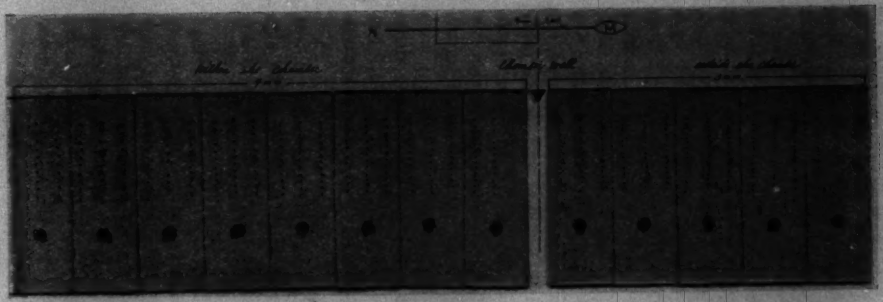

Fig. 4.

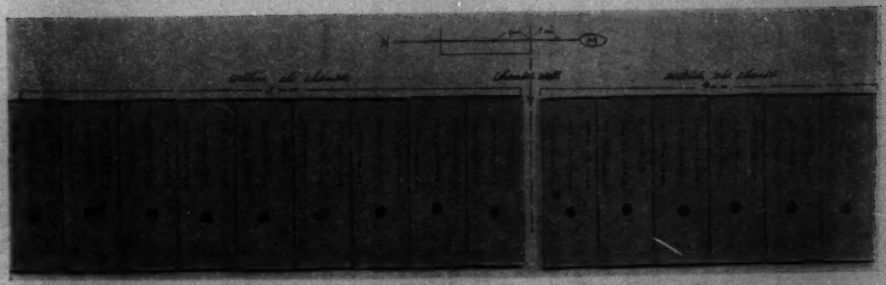

Fig. 5.

Whether the result B is due to the diffusion factor or to "transitional decrement" is a problem of much interest, although Hayashi's results offer important evidence in favour of the diffusion factor. To answer this question we have chosen dye solution and used it with or without narcotic instead of using a simple narcotising solution alone, because by this means we can judge the distribution of the dye within the nerve by the depth of its staining.

Exp. 1. The nerve muscle preparation *NM* was drawn through the narcotising chamber in the usual way as we do in narcotising chamber experiment (see Fig. 4). The chamber (with 1 mm. thick wall) was then filled with 2·0–2·5 p.c. urethane-Ringer's solution which contains 1·0 p.c. methylene blue or 2·0 p.c. carmine. After the conduction failed from the outside electrode, the part of nerve near the edge of the narcotising chamber was cut in serial transverse (or longitudinal) sections, each piece being 15–18 microns thick. One example in which carmine was used as a dye is given in Fig. 4. It will be seen that, on one hand, the dye diffuses out of the chamber more than 3 mm. and on the other hand, the nerve inside the chamber is not stained in equal depth. The colour becomes gradually deeper as the distance from the chamber wall increases until it reaches a full depth at about 3·5 mm. from the chamber wall. From numerous experiments made in this way with various concentration of the dye, it was found that the distance within which the influence from the normal part (*i.e.* gradient of narcosis) can be detected in the chamber becomes gradually greater as the concentration of the dye is decreased.

Another example is given in Fig. 5 in which methylene blue is used as a dye. Fig. 6 shows a longitudinal section of the nerve at the edge of the narcotising chamber. We see the similar diffusion phenomenon here as in the transverse section.

Exp. 2. Two nerve muscle preparations taken from the same toad were passed through the same narcotising chamber such as shown in Fig. 7, so that one nerve was exposed to the narcotising solution (containing dye) for a length of 20 mm., and the other for a length of only 4 mm. (below the "limit-length"). At the stage of narcosis at which the conduction failed in the longer region—the conduction does not yet fail in the shorter region, because it is below the "limit-length"—the two middle parts (*m* and *n*) of the both narcotised regions were taken out of the narcotising solution and were examined in serial transverse sections. It will be seen in Fig. 7 that the middle part *m* in the shorter stretch is not so deeply stained as the middle part *n* in the longer

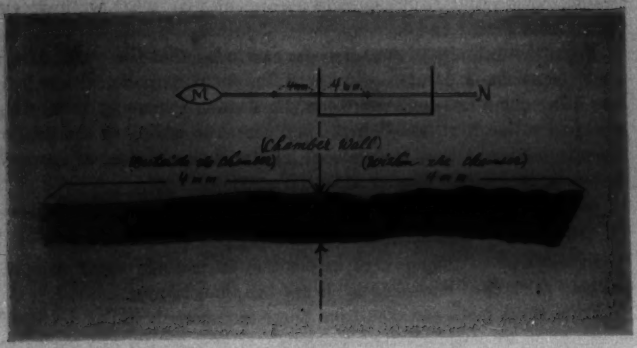

Fig. 6.

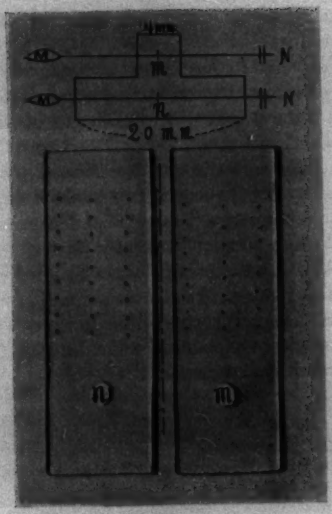

Fig. 7.

stretch. This result is in complete agreement with what we have expected from Figs. 1 and 3.

From these results it can safely be concluded that the qualitatively same phenomenon occurs as to the diffusion of narcotic, although it may differ quantitatively. Moreover, H. Tamura made an interesting observation that the "limit-length" is different according to the kind of narcotic used and that it increases as the concentration of narcotic decreases, for instance in case of the chloretone

0·5 p.c. ... 4 mm.	0· 2 p.c. ... 8– 9 mm.
0·3 p.c. ... 6 mm.	0·12 p.c. ... 10–11 mm.

In the case of abolition of conduction in isotonic sugar solution (without salt), the "limit-length" is not less than 15 mm.

From these facts and also from the experimental results described elsewhere(2), we conclude that the result B in question can easily be explained by the diffusion factor alone, without assuming "transitional decrement."

Summary.

The experiments in the narcotising chamber show two kinds of results:

A. The time required for suspension of conduction is not dependent upon the length of narcotised nerve, if its length is longer than 6 mm.

B. The time is dependent upon the length of narcotised nerve, if its length is shorter than 6 mm.

The result A represents the non-decremental conduction in narcotised region. And as to the result B two kinds of explanation are suggested; a diffusion factor and a "transitional decrement." To determine this alternative a dye solution was used instead of using a simple narcotising solution, and the part of nerve near the edge of the narcotising chamber was examined in serial transverse or longitudinal section. From the microscopical preparations thus made it was concluded that the result B can easily be explained by the diffusion factor alone, without assuming "transitional decrement."

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MORE REFLEX EFFECTS OF ACTIVE MUSCULAR CONTRACTION.

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(*From the Physiological Laboratory, Oxford.*)

Introduction.

In an earlier communication(4), it was shown that proprioceptive impulses set up by active contraction of the cat's hamstring muscles readily inhibit decerebrate tonus in the quadriceps extensor muscle of the same side. The results[1] were sufficiently interesting to make it desirable to repeat the experiments with more delicate means of recording, and to extend them if possible to other pairs of muscle groups.

This paper describes the results obtained by the use of a high-frequency isometric myograph with optical recording system of the type used by Sherrington in recent work and described in detail by Fulton(7). The appropriate spinal motor root to the "exciting" muscle was stimulated by a coreless Berne induction coil fed by 2 volts and delivering about 50 double shocks per second. The shadow of a short-circuiting key in the secondary circuit was thrown on the slit of the camera by the same source of light as was that of the myograph pointer.

The operative procedure was exactly as described before subject to slight modifications (mentioned below) when different pairs of test muscles were used. The most satisfactory form of pelvic clamp we have found to be one which embraces the pubic crest in front and the apex of the pubic arch behind, *i.e.* its arms lie in an antero-posterior plane. With the ordinary type passing between the obturator foramina, there is much greater risk of the pelvis rotating slightly about the fixed head of the femur during strong contraction of the hamstrings. As an additional precaution against this source of error, the rectus femoris muscle has

[1] Since that paper appeared, some observations published by Osborne in 1924(16) have been brought to our notice. He elicited reflexes (rise of blood-pressure and movements of skeletal musculature) by stimulation of the peripheral stump of the cut facial nerve in anæsthetised dogs, and attributes them to excitation of the proprioceptive end-organs in the facial muscles when the latter are thrown into contraction.

been cut across at its pelvic attachment in experiments involving the use of quadriceps extensor. The tendon of the "reacting" muscle was connected with the myograph by a light steel hook.

In experiments where inhibition of tonus was to be recorded, it was necessary to have the muscle under considerable tension at the beginning of each observation. This end was secured by using a table with a rotating top. The cat lay on its left side with the reacting muscle pulling horizontally on the myograph. By rotating the table top and fixing it rigidly just before an observation, any degree of initial stretch could be imposed.

In the experiments previously described, a knee flexor (hamstrings) and a knee extensor (quadriceps) were the exciting and reacting muscles respectively. In the present series, the following pairs, including synergic as well as antagonistic muscles, have also been studied: knee extensor (vastocrureus) acting on knee flexor (semitendinosus); knee flexor (hamstrings) acting on hip flexor (sartorius); ankle flexor (tibialis anticus) acting on hip flexor (sartorius); knee extensor (vastocrureus) acting on knee extensor (vastocrureus) of the opposite side; knee flexor (hamstrings) acting on knee extensor (quadriceps) of the opposite side. A technical limit is set to the number of pairs that can be investigated, by the condition that the motor root used for stimulating the exciting muscle must supply no fibres to the reacting muscle. The extensive overlap in their segmental nerve supply thus prevents the use of such obvious pairs as ankle flexor and ankle extensor, or biceps femoris and semitendinosus.

Effect of hamstrings on vastocrureus.

(1) The hamstrings of decerebrate cats were made to contract by faradic stimulation of the 7th lumbar or 1st sacral ventral roots, and the ensuing changes in tension of the patellar tendon were recorded. Except hamstrings and quadriceps extensor of the right side, all the muscles of both hind limbs were paralysed by nerve section. In most of the experiments rectus femoris was detached from its pelvic origin, so that the only structures connected with the patella were attached above to the femur. Other operative details are described in full in the previous communication (4).

The results of more than 30 experiments amply confirm our previous findings that active contraction of the hamstrings reflexly inhibits tonus in vastocrureus and that this effect is more marked when the former muscles are prevented from shortening. Suitable control experiments have been carried out and there is no need to dwell further on this

observation. The present paper deals with some features of the isometric record of this inhibition.

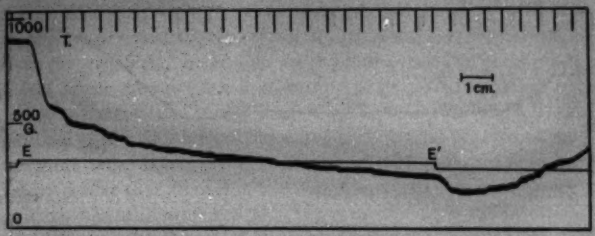

Fig. 1. Quadriceps extensor of decerebrate cat. Hamstrings made to contract by stimulation of 1st sacral motor root between *E* and *E'*. Coil distance 26 cm. Tendon movement magnified ×65 in original record. Tension shown by scale on left. *T* = time in 0·1 sec.

As shown in Fig. 1, there is frequently "an initial plunge of a steepness much greater than that of the later course of the decline," to quote Liddell and Sherrington's account(15) of the effect of weak inhibitory stimulation during the course of a crossed extension reflex. This rapid onset is not invariable and tends to disappear when weaker stimuli are applied to the motor root to hamstrings. A comparable phenomenon has been noticed by a number of observers in excitatory reflexes(5). The ensuing "inhibitory recruitment" may be very gradual and prolonged. In Fig. 1 the tension is still falling at the end of 2½ sec. of stimulation, while in some experiments relaxation has not been complete in 5 sec. When the stimulus is cut off there is nearly always a further decrease in the tension of vastocrureus, this secondary fall occupying not more than 80σ. It is followed by a greater or less degree of recovery towards the previous resting tension. The recovery, like the initial fall, often occurs in a series of steps. Sometimes there is no recovery and the muscle is left in an atonic condition. Very rarely, marked rebound contraction brings the tension to considerably more than its previous resting level. Fig. 2 shows the beginning (220σ after the "off" signal) of such a rebound contraction which in the next few seconds subsided to the resting tension. The occurrence of recovery and occasional rebound is interesting because inhibition of the myotatic contraction of quadriceps by passive stretch of a knee flexor was found by Liddell and Sherrington(14) to be followed by almost no recovery.

The interval between the application of the stimulus to the motor root

and the beginning of relaxation in the extensor muscle, varies considerably in our experience in different animals, and even in successive

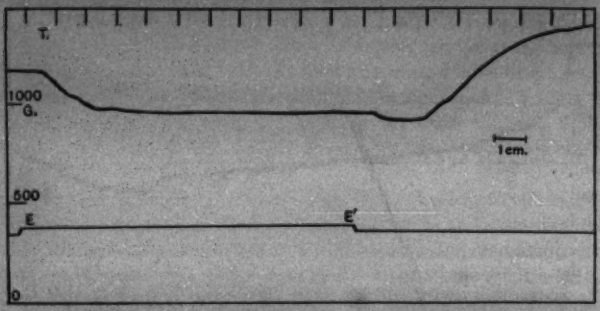

Fig. 2. Quadriceps extensor of decerebrate cat. Hamstrings made to contract by stimulation of 7th lumbar motor root between E and E'. Coil distance 24 cm. Tendon magnification $\times 65$. T = time in 0·1 sec.

observations with varying strengths of stimulus during the same experiment. The mechanical latency tends to become shorter as the intensity of stimulation is increased. This is perhaps to be expected in reflexes showing recruitment and was noted by Liddell and Sherrington in the crossed extensor reflex ((12), p. 325). In our records the mechanical latency is rarely less than 50σ or more than 85σ. In experiments where reflexes have also been recorded from ipsilateral and contralateral afferent nerves, it is uniformly longer than that of the former, and as a rule slightly shorter than that of the latter.

For the elicitation of the reflex in an active preparation, the stimulus must be somewhat, but not greatly, stronger than is required to produce a minimal contraction of the hamstrings. As the stimulus is made weaker, the initial sharp fall in tension tends to disappear and the slope of the whole curve becomes less steep. The terminal relaxation also becomes less marked.

(2) The surprising feature of these myograms is the final decline in tension on withdrawal of the stimulus. At first sight one is reminded of the very similar isotonic tracings published by Graham Brown ((10); see also (11)) of the inhibition of tonus in the decerebrate cat's gastrocnemius muscle during and after an artificially evoked ipsilateral flexion reflex. He described the incremental relaxation after the termination of the stimulus as "rebound relaxation after inhibition." Liddell and

Sherrington(13) further investigated the same phenomenon in the quadriceps muscle on stimulating an ipsilateral afferent nerve during the course both of a stretch reflex and of a crossed extension reflex. Their experiments clearly show "that whether reflex contraction of the muscle be in process or not at the time when the ipsilateral afferent is stimulated, this nerve regularly, in certain experiments, unfolds concurrently a two-fold effect on the muscle, namely a large inhibitory effect (which requires a contraction background to make it evident) and a smaller excitatory effect. This latter documents itself independently and in spite of the inhibitory. It sets in quicker than and, on cessation of the stimulus, subsides earlier than the inhibitory. Hence, where this excitatory effect is present, the inhibitory relaxation is precluded from full completeness by it, but on withdrawal of the ipsilateral nerve-stimulation, the inhibition, owing to its outlasting the excitatory contraction, produces the full relaxation of its pure inhibitory component" (p. 227).

There are, however, certain objections to accepting this explanation of the final fall in our experiments. (*a*) Instead of stimulating electrically the trunk of a "mixed" afferent nerve containing both excitatory and inhibitory fibres for the reacting muscle, we were applying what we believe to be the normal stimulus, viz. active muscular contraction, to what is probably a homogeneous group of proprioceptive end-organs in the hamstring muscles. It is hardly to be expected that of the impulses ascending to the central nervous system in response to any natural stimulus acting alone, some will cause excitation and others inhibition in a single effector organ ((14), pp. 280–283; (24)). (*b*) There is no other indication in our records of any excitatory element. We have indeed occasionally found a small increase of tension in the test muscle immediately following the hamstring contraction and before the relaxation begins. But this has always been within the limits of movement allowed by faulty fixation of the pelvis, and further evidence of its purely mechanical causation is afforded by its short latency. In many of the records there is no sign of it, and in the atonic muscle (*e.g.* when a second stimulus is applied to the motor root to the hamstrings of a preparation in which no recovery has succeeded the inhibitory relaxation caused by a previous stimulus) the myograph shadow remains stationary provided the pelvic clamp is efficient. (*c*) In two experiments we have administered strychnine intravenously, beginning with 0·09 mg. per kilo. body weight and later increasing the dose until mild convulsions appeared. Subsequent contraction of the hamstrings showed no reversal of reflex effect on vastocrureus; on the contrary, the relaxation of the latter muscle was

more marked than before, because its initial tonus was greater. Liddell and Sherrington(14) similarly obtained no "strychnine reversal" of the inhibitory effect which passive stretch of a knee flexor exerts on the stretch reflex of quadriceps. Adopting the view that strychnine acts by augmenting excitatory reflexes disproportionately to inhibitory (17, 3, 14), these observations provide strong evidence that there is no excitatory element in the reflex under discussion.

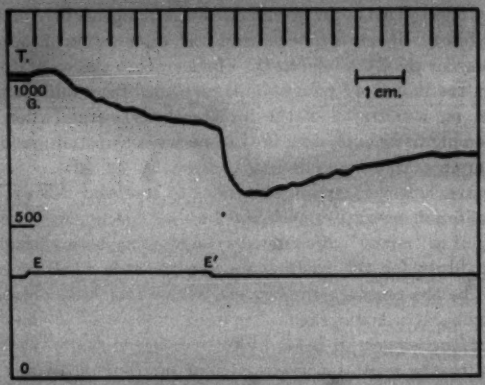

Fig. 3. Quadriceps extensor of decerebrate cat. Hamstrings made to contract by stimulation of 1st sacral motor root between *E* and *E'*. Coil distance 26 cm. Tendon magnification × 65. *T* = time in 0·1 sec.

There is, of course, the possibility that the final fall in tension is a mechanical artefact due to imperfect fixation of the attachments of the reacting muscle during hamstring contraction. We have tried to guard against this by frequent testing of the fixing apparatus, and when the phenomenon is so marked as in Fig. 3, such an explanation seems impossible. The following facts should also be borne in mind. (*a*) The constancy of the phenomenon contrasted with the relative infrequency of other evidence of inadequate fixation (*e.g.* initial contraction—shown in Fig. 3, but minute compared with the final fall). (*b*) It begins as a rule 50 to 60σ after the "off" signal. In cases where there is reason to suspect a mechanical artefact, relaxation begins in our experience not more than 40σ after the signal. (*c*) It seems to make no difference whether or not rectus femoris is left attached to the pelvis. (*d*) The absence of any move-

ment of the myograph in several experiments when the vastocrureus is either toneless or is paralysed by nerve section.

To summarise, there is evidence that the final fall in tension immediately following withdrawal of the stimulus is a true physiological event and not a mechanical artefact. There are serious difficulties in regarding it as an expression of simultaneous excitation and inhibition, in which the latter component outlasts the former. In other words, it is not comparable with the superficially similar records which are obtained when a "mixed" afferent nerve is stimulated, and which have been investigated by other observers. At present we are unable to offer any adequate explanation.

(3) So far, inhibition of postural tonus or stretch reflex only has been described. In a further series of experiments we have found that the contraction of vastocrureus in a crossed extensor reflex (decerebrate cat) is also inhibited by hamstring contraction. Weak electrical stimulation of the left sciatic nerve with a second induction coil was used to give the back-ground reflex in the right vastocrureus. During the course of the

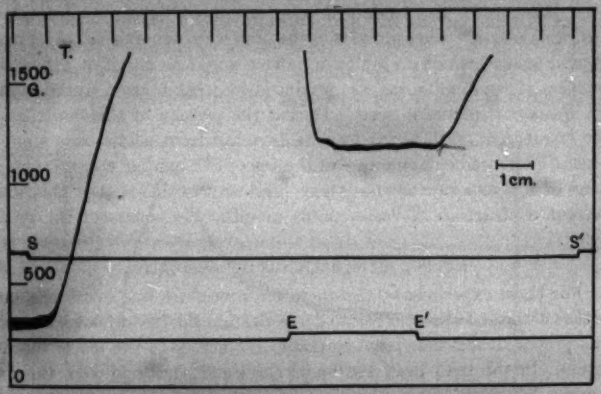

Fig. 4. Vastocrureus of decerebrate cat.
S to *S'* = stimulation of contralateral sciatic nerve. Coil distance 23·5 cm.
E to *E'* = stimulation of 1st sacral motor root causing contraction of hamstrings. Coil distance 22 cm. Tendon magnification × 65. *T* = time in 0·2 sec.

contraction so evoked, the right hamstrings were made to contract by intercurrent faradic stimulation of the 1st sacral motor root. According

to the relative strengths of the two stimuli, the contraction is either partially or completely inhibited. Figs. 4 and 5 were obtained in separate preparations.

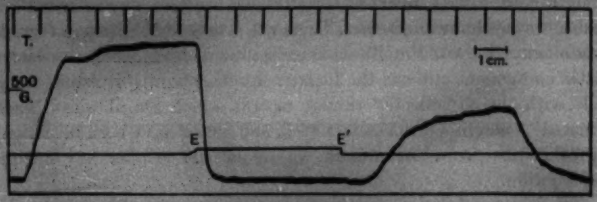

Fig. 5. Quadriceps extensor of decerebrate cat. Record similar to Fig. 4, but from different experiment. Contralateral sciatic nerve, coil distance 20 cm. 1st sacral motor root, coil distance 17 cm.

Effect of vastrocrureus on semitendinosus.

In four preparations with the spinal cord transected at the level of the 12th thoracic vertebra prior to decerebration, myographic records have been taken from the tendon of semitendinosus during contraction of vastocrureus. All other muscles in the limb were denervated. As a background against which an inhibitory effect would be manifest, reflex contraction of semitendinosus was produced by weak faradic stimulation of the ipsilateral peroneal nerve. During the plateau of this contraction, the quadriceps (with rectus femoris detached from pelvis) was made to contract by faradic stimulation of the 4th or 5th lumbar ventral root. In none of the experiments has there been any evidence that this intercurrent contraction of vastocrureus modifies the course of the flexion reflex.

Effect of hamstrings on sartorius.

For these experiments, the operative procedure was much the same as that described above. Prior to decerebration the 7th lumbar motor root was prepared and the spinal cord was cut across in the lower thoracic region. In the limb itself the hamstrings and sartorius were the only muscles left with nerve supply intact. When taking records from sartorius it was found especially important to ensure that the small twigs of the sciatic nerve distributed to quadratus femoris and adjacent muscles were cut, and that all branches of the gluteal nerves were divided as they emerged through the sciatic notch. Merely cutting across the muscles supplied by them was not sufficient to abolish the effects of the contraction on sartorius. The hamstring muscles were not dissected out

or disturbed in any way and were made to contract by electrical stimulation of the 7th lumbar or 1st sacral ventral spinal roots. The lower back muscles were cut freely to prevent arching of the back during stimulation of the root.

The sartorius was attached to the myograph by the cut patellar tendon from which the denervated quadriceps extensor was detached close above the patella. By careful dissection sartorius was freed on its deep surface from quadriceps and tensor fasciæ femoris, and on its superficial surface, near its origin from the pelvis, all bands between it and the abdominal muscles were divided. Its medial edge was separated from surrounding structures as far proximally as was compatible with the preservation of its blood supply. By these means the muscle was well isolated, and during an experiment care only was required to prevent cooling and drying. The medial part of the muscle, which is inserted into the tibia, was of course not recording, but in the cat a large proportion of the fibres are inserted into the patella. The limb was rigidly fixed to the table top by drills in both ends of the femur and an antero-posterior pelvic clamp.

That the reflex activity of the sartorius was not seriously impaired by the operation was shown by the occurrence of a "jerk" in a few preparations on tapping the table top. As this reflex has, it is believed, not previously been described in this muscle, a record of it is reproduced (Fig. 6).

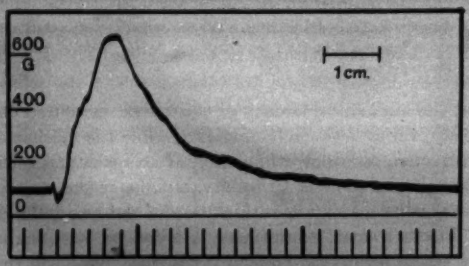

Fig. 6. Sartorius of spinal cat. Jerk excited by tapping table. Tendon magnification ×45. Time in 0·02 sec.

The myograph tracing shows a small increase of tension of brief duration due to the tap, followed after 10σ by the reflex contraction. This mechanical latency is about the same as that shown in comparable plates of the decerebrate knee jerk by Fulton and Liddell(8) and Ballif, Fulton, and Liddell(2), and of the supraspinatus jerk by Denny-Brown and Liddell(6). It is rather longer than was reported for the anæsthetised rabbit's knee

jerk by Waller (29) and by Gotch (9), who used a cruder method of recording. The myogram shows clearly that the response is not a simple twitch, but an asynchronous and probably repetitive discharge of the motor neurones concerned (1, 2, 3). Tendon jerks are notoriously difficult to elicit in the flexor muscles of the cat, and the only one that has been investigated in detail is that of tibialis anticus (1). We have been quite unable to record any jerk from semitendinosus, though as noted by Liddell and Sherrington (14) it is frequently possible to feel a momentary contraction of the muscle on plucking its tendon. Sternberg (28) and Viets (25) found the knee jerk more easily elicitable during stimulation of an afferent nerve of the contralateral limb, but in our experience stimulation of an ipsilateral nerve does not increase the excitability of the proprioceptive reflex arc of semitendinosus sufficiently for a jerk to be obtainable. This is surprising in view of the occurrence of hamstring jerks in normal man, where they are constant enough to be of clinical importance.

When a motor root supplying the hamstring muscles of such a preparation is stimulated, contraction of the hamstrings is followed by a well-marked reflex contraction of the sartorius (Fig. 7). With our

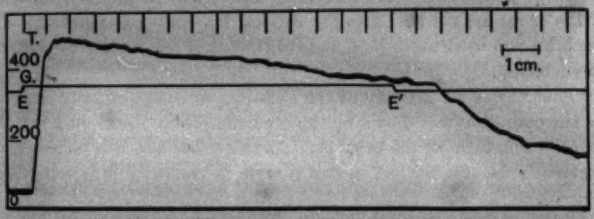

Fig. 7. Sartorius of spinal cat. Hamstrings made to contract by stimulation of 7th lumbar motor root between E and E'. Coil distance 20 cm. Tendon magnification $\times 45$. T = time in 0·1 sec.

apparatus, the mechanical latency of this reflex, measured on a rapidly moving plate, varies between 35 and 42σ; while the mechanical latency of the contraction following stimulation of an ipsilateral afferent nerve in the same preparations is 16 to 25σ. In the record reproduced, the tension reaches its maximum within 100σ of the beginning of the contraction. In other cases, recruitment is found to be much slower and 400σ may elapse before the plateau is reached. Similarly the duration of after-discharge varies. In many observations relaxation is not complete 1 sec. after cessation of the stimulus.

In previous experiments (*supra* and (4)), the reflex effects of hamstring contraction were shown to be greater when these muscles were prevented from shortening. This is also true of the effect on sartorius. In one preparation, in which the 7th lumbar motor root was stimulated at

coil distance 20 cm., the tension developed by the sartorius in four successive observations was as follows:

	grm.
Knee allowed to flex	340
Knee prevented from flexing	430
Knee prevented from flexing	450
Knee allowed to flex	325

In the same cat, contraction of the sartorius was observed on mere passive stretch of the hamstrings. Magnus(29) states that in the intact or thalamic animal's fore-limb passive stretch of one digit flexor causes reflex contraction in the whole group of flexor muscles in the forearm.

The effects described above were abolished by cutting the nerve to hamstrings and could not therefore be due to spread of current from stimulating electrodes to neighbouring posterior roots. That they were not due to purely mechanical pull from the hamstring muscles was proved by frequent testing of the fixing apparatus and by direct inspection of the contracting fibres of sartorius. Even with the most careful fixing, slight rotation of the pelvis about the head of the femur could never be absolutely prevented, but it could be reduced to negligible proportions. In the next series of experiments this source of error does not arise, since tibialis anticus is far removed from the pelvis.

Effect of tibialis anticus on sartorius.

In the decerebrate cat made spinal by transection of the cord in the lower thoracic region, all the muscles of the hind limb were denervated on the right side with the exception of sartorius and tibialis anticus. The peronei muscles and extensor digitorum longus were cut through below the knee to expose the breaking up of the peroneal nerve into its terminal branches. All of these except that going to tibialis anticus were severed. The annular ligament of the ankle was divided and the tendon of tibialis

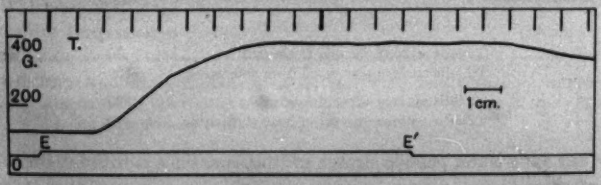

Fig. 8. Sartorius of spinal cat. Tibialis anticus made to contract by stimulation of 7th lumbar motor root between *E* and *E′*. Coil distance 20 cm. Tendon magnification ×45. *T* = time in 0·02 sec.

anticus separated from surrounding structures before the preparation was set up, in order to facilitate the cutting of the tendon at a later stage of the experiment. The preparation of sartorius and the fixation of the limb and pelvis to the table were exactly as described in the last section.

On causing tibialis anticus to contract by stimulation of the 7th lumbar motor root, sartorius responded by a contraction in each of the nine animals which we have employed. The mechanical latency was as a rule between 32 and 42σ; in one preparation it was as long as 54σ, and in another as short as 20σ. It was always notably longer than that of the similar response to stimulation of an ipsilateral afferent nerve or sensory root. Thus in one experiment, the following records were made in rapid succession employing the same strength of stimulus throughout.

		Mechanical latency (σ)
1. 7th lumbar motor root	Ankle flexion and sartorius contraction	50
2. 7th lumbar motor root	Ankle flexion and sartorius contraction	50
Peroneal nerve cut		
3. 7th lumbar motor root	No ankle movement; nil in sartorius	
4. 7th lumbar motor root	No ankle movement; nil in sartorius	
5. Popliteal nerve	Sartorius contracts	28
6. Peroneal nerve	Sartorius contracts	30

After ligating the tendon of tibialis anticus at its junction with the fleshy part of the muscle, and detaching it from the bone below (thereby abolishing all movement of the ankle joint), there was no obvious change in the reflex effect on sartorius provided the muscle was prevented from shortening. If shortening were permitted, the contraction of sartorius was generally smaller. The following are two examples of such pairs of observations.

	Tension developed by sartorius (grm.)
Tibialis anticus allowed to shorten	35
Tibialis anticus prevented from shortening	100
Tibialis anticus allowed to shorten	330
Tibialis anticus prevented from shortening	450

In our experience, passive stretch of tibialis anticus is without effect on sartorius.

If all other muscles of the limb have been satisfactorily denervated, section of peroneal nerve abolishes the reflex. Mechanical factors are

excluded (*a*) by the impossibility of tibialis anticus exerting any pull on sartorius or its attachments, (*b*) by the perfect steadiness of the myograph after paralysing sartorius by cutting its nerve, and (*c*) by the fact that contraction of some fibres in sartorius is clearly visible on inspection of the muscle.

EFFECT OF VASTOCRUREUS ON VASTOCRUREUS OF OPPOSITE SIDE.

In the decerebrate preparation the nerve to quadriceps in the left leg has been left uncut in some experiments in which the only other muscles of either hind limb with nerve supply intact have been quadriceps and hamstrings of the right leg. Records were taken, as described above, of the changes in tension of right vastocrureus. On making the left vastocrureus (rectus femoris being detached from the pelvis) contract by stimulation of the peripheral stump of the cut left 6th lumbar ventral root, we have recorded well-marked relaxation of the right vastocrureus, sometimes followed, on withdrawal of the stimulus, by rebound contraction.

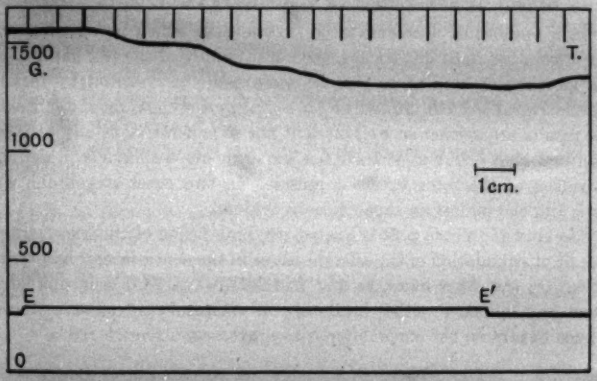

Fig. 9. Right vastocrureus of decerebrate cat. Left vastocrureus made to contract by stimulation of 6th lumbar motor root between *E* and *E′*. Coil distance 22 cm. Tendon magnification × 65. *T* = time in 0·02 sec.

It has long been known that the proprioceptive fibres from the extensor muscles are of two kinds (23). One set carries impulses which reflexly induce shortening of the very muscle in which they originate (tonus, stretch reflex, knee jerk, "shortening reaction"), and at the same time

cause "reflex relaxation (more rarely reflex contraction) of the fellow muscle of the opposite limb" (Sherrington, (22)). The other set carries impulses which reflexly inhibit the muscle in which they originate (20, 21). In the "lengthening reaction," the end-organs of this set respond to the same kind of stimulus, differing perhaps in degree (27), as sometimes excites the former set, i.e. they respond to passive "stretch of the tonic muscle: this stimulus causes reflex lengthening of the muscle itself, and reflex contraction of the fellow muscle of the opposite limb" ((22); Philippson's reflex, (18)).

In our experiments, it is evidently the endings of the former set that have been stimulated by active contraction of vastocrureus. In reflex stepping, therefore, the act of extension in one limb will initiate a volley of centripetal impulses tending reflexly to facilitate simultaneous flexion of the opposite limb. During the flexion phase, possibly the passive stretch of the knee extensor may evoke Philippson's reflex and so assist in the simultaneous extension of the opposite limb.

Effect of hamstrings on quadriceps of opposite side.

In a number of decerebrate preparations in which myograms were taken from the right quadriceps, the nerve supply of the left hamstrings was kept intact, and these muscles were made to contract by faradic stimulation of the 7th lumbar or 1st sacral motor roots on the left side. The results are neither so well marked nor so conclusive as any of those described above, but in at least five experiments we have seen definite relaxation of the tonic extensor muscle. In two cases strychnine was given and the inhibition became more evident.

Sherrington ((19), p. 564) has reported inhibition of the knee jerk as a result of stimulation of the afferent fibres in the contralateral hamstring nerve. On the other hand, he and Liddell ((14), p. 283) were unable to detect any influence, either inhibitory or excitatory, of passive stretch of knee flexors on the contralateral knee extensor's stretch reflex.

Summary.

1. Isometric records have been taken, from the cat's knee extensor muscle, of the inhibition of decerebrate tonus, which results, as described in a previous communication (4), from active contraction of the knee flexors.

2. The tracings show a sharp initial fall in tension, followed by slow inhibitory recruitment and a further relaxation when the stimulus is

withdrawn. Recovery may or may not occur, and is very occasionally accompanied by rebound contraction. The cause of the terminal relaxation is obscure.

3. Strychnine does not reverse the inhibitory effect.

4. The reflex contraction of vastocrureus produced by stimulation of a contralateral afferent nerve (crossed extension reflex) is similarly inhibited when, by stimulation of an appropriate ventral spinal root, the hamstrings are concurrently thrown into contraction.

5. On the other hand, reflex contraction of semitendinosus (ipsilateral flexion reflex) is uninfluenced by concurrent contraction of vastocrureus.

6. Active contraction of hamstrings (knee flexor) or of tibialis anticus (ankle flexor) sets up a volley of centripetal impulses which cause reflex contraction of sartorius (hip flexor). Prolonged recruitment and after-discharge are features of this reflex. The reflex is best seen when the "exciting" muscle is prevented from shortening. In tibialis anticus the proprioceptive end-organs concerned are situated in the fleshy part of the muscle.

7. Active contraction of vastocrureus causes inhibition of tonus in the fellow muscle of the opposite limb. The possible bearing of this on reflex stepping is indicated.

8. Active contraction of hamstrings probably causes inhibition of tonus in vastocrureus of the opposite limb.

9. The occurrence of a sartorius "jerk" in spinal cats is reported and illustrated.

It is a pleasure again to express our thanks to Sir Charles Sherrington for much useful advice.

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THE INTERRELATION OF NUMBER, VOLUME, DIAMETER AND AREA OF MAMMALIAN ERYTHROCYTES. By W. F. EMMONS.

(From the Laboratory of Physiology and Experimental Medicine, McGill University, Montreal, Canada.)

Much attention has been given to the variations in volume of the red blood corpuscles under both normal and pathological conditions. What determines the size of the red cell for different species of animals is yet unknown; certainly there is no relation between the size of the animal and that of its erythrocyte. Rice(1) finds that the particles of a lecithin emulsion in water assume the same form as the mammalian red blood cells, being circular discs which are dumbbell-shaped in cross-section. It is of interest to note that the range of variation of the diameters of the particles he observed coincides with that found in different species of the mammalian class, viz. 5 to 10μ. In view of their non-nucleated character one might suspect from these findings of Rice that the red cells are rather inert bodies, whose form and dimensions depend upon the physical forces at work in the medium in which they are suspended.

Be this as it may, the cell dimensions are curiously constant under definite physiological and pathological conditions. Capps(2) and also Haden(3) have compiled voluminous data to show that in pernicious anæmia the cell is nearly always enlarged in volume, while in other conditions the volume remains normal or may even decrease. Price-Jones(4) has shown the same thing to be true of the cell diameter in the dried smear. His findings are corroborated by Campbell(5) and by Grosh and Stifel(6).

Throughout the present paper the figures for cell diameter refer to the diameter of the cell as found in dried blood smears. The author is fully aware of criticisms levelled at the validity of such measurements and their acceptance as criteria of the cell dimensions when in plasma. Ponder and Millar(7, 8) claim that the diameter of the cell in plasma for man (no other species is mentioned) is 8·8μ, or just 1·0μ larger than in the dried state. They freely admit the many difficulties which must be contended with in making these observations, such as anticoagulant precautions, the tendency of the cells to rouleaux formation, dilution

of the blood with plasma, etc. They also cite possible sources of error, which are not a few. Among these may be mentioned alteration of plasma concentration involving change in osmotic pressure, variation in hydrogen-ion concentration through exposure to air, brownian movement causing enlargement of the photographic image, as well as the common photographic errors of resolution.

Excellent as the work of these authors on the cell in plasma may be, it cannot be regarded as final, owing to the many factors which tend to vitiate the constancy of the results. Opposed to these photographic determinations stands the work of Millar(9), who claims that his diffractometer method of measuring the cells shows no difference between the cell in plasma and in the dried state.

To eliminate the difficulty of rouleaux and still have a high cell concentration in the plasma, the present author applied Ponder's method to goat's blood, which cannot be induced to form rouleaux. Here the ratio of cell thickness to diameter is 1 : 2, whereas in man it is about 1 : 4. The cells may be allowed time to settle perfectly and the measurements are not hurried in the same way as Ponder describes in his experiments. Measurements of the diameter made under these conditions both with the eriometer (Emmons(10)) and by photography showed that in plasma it is actually smaller than in the dried state. It is then considered justifiable, in view of the many objections to measurements on the cells in plasma, to discard this method until more reliable work has been done upon it. Admitting the possibility of some slight error in measurements of the dried cell, the constancy of these determinations in cells from the same species, together with their great facility of measurement and the apparent interrelationship to be here described, indicate that this dimension is one which must be a very close approximation to the corresponding dimension of the cell as it exists in the circulating blood.

Since cell diameter is now so easily obtainable by means of the eriometer, it was of interest to know whether an increase in volume bears a constant relation to an increase in diameter. If so, the same diagnostic significance ascribed by Haden to increased cell volume might equally be ascribed to an increase in the average cell diameter. The present investigation was undertaken for the purpose of examining this question.

In studying the problem it was necessary to have cells of widely varying diameter and volume. Such cells are to be found in pathological conditions, but these are unsuitable because of their variation

in any one blood sample. Therefore healthy animals of different species were selected, whose blood provided a considerable range of cell dimensions combined with uniformity of size in each type. The species chosen were goat, cat, rabbit, dog and man.

In Addison's anæmia in man the average corpuscle volume may rise as high as 150 cu. μ or more. This is nearly a twofold increase over the volume of the normal cell (88 cu. μ). In the blood of the goat the average corpuscle volume is 24·6 cu. μ. Thus between goat, 24·6 cu. μ, and man, 88 cu. μ, we have a variation in normal animals much greater than that in pernicious anæmia. The cells of cat, rabbit and dog fall between these figures.

It was necessary to obtain normal standards for the erythrocytes of the animals selected. This involves a large number of individual determinations, the results of which are represented in Table I. Whenever alternative methods were available for checking any determination they were used. For example, the cell volume[1] was estimated in the following ways:

1. By the ordinary hæmatocrit.

Dry pulverised crystalline potassium oxalate was added to the fresh blood (10 mg. per 5 c.c. blood) to prevent clotting. There is no dilution by this method.

2. By the van Allen hæmatocrit.

Clotting is prevented by adding an isotonic anticoagulant fluid to a definite amount of blood. The cells are thrown down as before.

3. By the refractometer.

This method consists in estimating at constant temperature the change in concentration of the plasma proteins as indicated by the refractive index, when a unit volume of blood is diluted with normal saline to exactly twice its volume. The volume occupied by the cells (*i.e.* the non-dilutable portion of the blood) can be found by the following calculation:

If X = amount of plasma in unit volume of blood,
P = refractive index of plasma,
S = refractive index of saline,
M = refractive index of mixture,
$K = \dfrac{\text{refractive index}}{\text{protein concentration}}$;

then protein concentration in plasma $= (P - S)\,K$,

$$\text{protein concentration in mixture} = \frac{x}{x+1}(P-S)\,K = (M-S)\,K,$$

$$x = \frac{(M-S)}{(P-M)}.$$

[1] By "cell volume" is meant the percentage of any given volume of blood occupied by the cells themselves. The term "corpuscle volume" is used here to designate the volume of the individual corpuscle, which is determined by dividing the cell volume per cu. mm. by the number of corpuscles per cu. mm. Corpuscle volume is stated in cubic microns.

But amount of cells in unit volume of blood $= (1 - X)$,

$$\therefore \text{ p.c. of cells} = 100 - \frac{100\,(M - S)}{(P - M)}.$$

This method is free from the objection that the interstices between the packed cells enter into the calculation. Nor is any error introduced by excessive compression of the cell mass as in centrifugation.

4. By the blood sugar method[1].

The same principle is involved, namely the change in concentration of a constituent of the plasma when whole blood is diluted with normal saline, but in this case plasma sugar instead of plasma protein was determined. For estimation of the blood sugar the method of Folin and Wu was used. It is of interest here to state that the values obtained coincide very closely with those given by the refractometer method.

The number of cells per cu. mm. was found by means of the ordinary hæmocytometer. To assure accuracy the counts were repeatedly checked. The cell diameter was measured by the eriometer, which has been fully described and discussed in another paper (10). In cases where estimates were found to differ from published data particular care was given to the determinations in question. The figures exhibited in Table I, representing as they do the result of several determinations often checked

TABLE I.

	Goat	Cat	Rabbit	Dog	Man
No. of cells per cu. mm. $\times 10^{-6}$	15·0	9·0	6·8	6·25	5·0
P.c. vol. of cells in blood	38·0	38·5	42·5	43·0	44·0
Corpuscle volume, μ^3	24·6	43·0	63·0	69·0	88·0
Corpuscle diameter, μ	4·0	5·6	6·6	7·2	7·8
Corpuscle maximum thickness, μ	—	2·1	2·15	1·95	2·05
Corpuscle average thickness, μ	1·95	1·75	1·84	1·70	1·84
Corpuscle area, μ^2, calculated by Guldin's Theorems	—	81·7	110·6	120·8	144·8
Area cylinder, similar volume and diameter, μ^2	49·73	79·94	106·57	119·72	140·63
Area sphere, similar volume, μ^2	40·9	59·3	76·5	81·3	95·6
Cell area according to Bürker's formula, μ^2	25·1	49·3	68·4	82·7	95·6
Total cell area based on calculation as cylinders, sq. cm.	7·48	7·19	7·24	7·48	7·03
Total cell area (sq. cm.) per cu. mm. as calculated by Guldin's theorems	—	7·35	7·52	7·55	7·24
Hb p.c. (Sahli scale)	90·0	90·0	90·0	90·0	90·0

by independent methods, are probably more accurate than those hitherto available. When reliable data were obtained for the number, the volume and the diameter of the corpuscles, a comparison of the results in the five different species suggested certain interesting relations between them.

If the corpuscle volume is plotted against the corpuscle diameter in

[1] This method was suggested to me by Mr W. H. Finney, of this Laboratory, who also carried out the determinations.

each species, the points obtained are found to lie as in Fig. 1. Suppose for the moment we regard the cells as short cylinders, then the following relation obtains between volume, diameter and height respectively,

$$H = \frac{4V}{\pi D^2}.$$

When in these (theoretical) cylindrical corpuscles we calculate the height for the experimentally obtained values of volume and diameter,

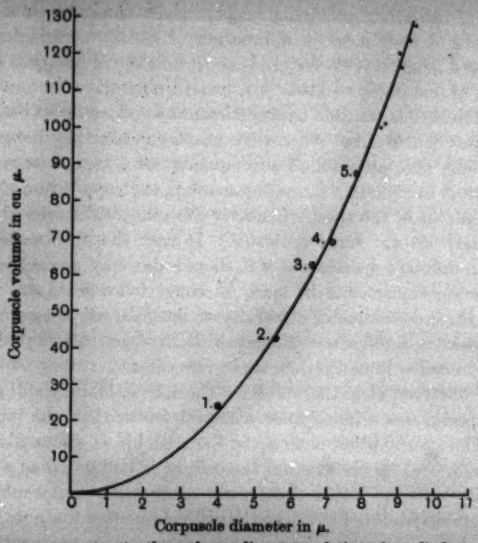

Fig. 1. The curve represents the volume diameter relations in cylinders of constant height (1·8 μ). Points 1 to 5 represent the empirical findings for the cells of goat, cat, rabbit, dog and man respectively. The dots beyond this apply to cells of pernicious anæmia in man.

we find in the case of the goat, 1·95 μ; of the cat, 1·75 μ; of the rabbit, 1·84 μ; of the dog, 1·70 μ; and of man, 1·84 μ. In other words, although volume and diameter vary markedly in the four species, the average thickness of the ideal or cylindrical cell is relatively constant and approximately equal to 1·8 μ. Of course the actual cells are not sections of cylinders, but it is of interest to note that when we insert the empirical findings of volume and diameter for the enlarged cells of pernicious anæmia in man, represented by a cluster of six dots at the

upper end of the graph, the same parabolic curve is found to pass through the cluster. The close correspondence between the measurements of the actual cells of goat, cat, rabbit, dog and man, including human pernicious anæmia cells, with the parabolic graph representing cylinders of varying cross-diameter but of uniform height, is certainly striking. When we consider that to a first approximation the blood cells do resemble truncated cylinders, we are forced to conclude that the thickness of the actual cells may be practically the same in each case. The volume of a cylinder is a function of the two variables, cross-diameter and height. If the height is constant the volume then becomes a function of one single variable, viz. cross-diameter.

Fortunately it is possible by experiment to make a direct estimate of the thickness of the cells. When they form rouleaux they become piled up like coins, and, as we shall subsequently see, measurements made upon the cells in rouleaux formation confirm the supposition that their thickness in four of the species is nearly the same. (The cells of the goat unfortunately do not form rouleaux.) If now the thickness remains constant in different species and if it should also stay unchanged in the cells of pernicious anæmia in man, it would follow that an observed change in the cross-diameter of pernicious anæmia cells would carry the same diagnostic significance as has hitherto been attributed to the alteration in cell volume that occurs in this disease.

It was observed that the value for the hæmoglobin (Sahli scale) in the four species was a much more constant factor than the percentage of cells. The presumption is that the hæmoglobin is either distributed uniformly throughout the mass of the cell, or is laid down as a layer at or near the surface. Under the former alternative one would expect the hæmoglobin reading to vary as the cell volume (see footnote, p. 217); under the latter alternative it might be expected to vary rather with the total surface area. In the five species here dealt with the maximum variation in cell volume was found between the blood of goat and man. This variation amounts to approximately 15 p.c., or well beyond the experimental error for the determination of cell volume which is not greater than 1 p.c. (see refractometer method, p. 217). This discrepancy between the hæmoglobin content of the blood and the cell volume in these species led one to look for some other factor in common which might prove to be more constant than the cell volume. Consequently it became of interest to devise an accurate method of estimating the total cell area per unit volume of blood. As will be shown later, the variation in total cell surface between these five species amounts to

approximately 4 p.c. (including the experimental error). These results for area, then, are considerably nearer the constancy of hæmoglobin findings than are those for cell volume. They would therefore tend to favour a surface distribution of the hæmoglobin as opposed to its uniform distribution throughout the cell.

One means that might possibly be employed for the calculation of total cell area is that of Gorter and Grendel(11), who, estimating by Langmuir's method the total area of a monomolecular film of lipoid which had been extracted from the cells, and assuming that this lipoid had been disposed on the surface of the erythrocytes, found that the area of this monomolecular film bore a fairly constant ratio to the total cell surface as calculated by Knoll's formula. Their conclusion was that on the surface of the cells the lipoid molecules are stacked two deep. If it should be true that the erythrocytes are covered by a layer of lipoid molecules two thick, then it is plain that by recourse to Langmuir's method one might obtain a very accurate estimate of the total surface area. The conclusions of Gorter and Grendel, however, still await confirmation.

As a rule, the surface area of the cells has been calculated on the basis of certain simplifying assumptions with regard to their shape. Bürker(12), neglecting the thickness of the cell, uses the formula $\pi D^2/2$. Ponder(13) uses a formula in which the thickness is also taken into account. For the thickness of the corpuscles of different animals he has to rely on the direct microscopic measurements of Gulliver(14). As, however, he and Millar(15) in a simultaneous paper are at somewhat elaborate pains to point out that direct microscopic measurements are subject to considerable error, it is plain that he could himself have placed but little reliance on Gulliver's measurements. Nevertheless these are vital for the development of his calculations.

In accordance with the principle so familiar in colloidal chemistry whereby area increases on subdivision, one might imagine that the cat, possessing small but more numerous cells, would far exceed man as regards cell surface per cu. mm. of blood. In order to settle such a question one must necessarily have an accurate method of estimating the area of a single cell without disregarding any portion of its complicated outline. This would not be a difficult matter if one knew the exact form of the meridional cross-section. But as this has never been precisely established, this obstacle had first to be overcome.

An approximation to the area is given by calculating the area of cylinders of similar volume and diameter to that of the cell. The objection

to this is that the concavities—and consequently a certain amount of surface—are neglected. A much more accurate approach is offered in the application of Guldin's (or Pappus') theorems to the problem. One of these theorems enables us to obtain a close approximation to the real form of the cell in cross-section. With this established, the other theorem readily gives us the exact surface of the solid figure.

As stated in Williamson's *Integral Calculus* (16) Guldin's theorems read:

1. If a plane curve revolve around any external axis, situated in its plane, the area of the surface generated is equal to the product of the perimeter of the revolving curve by the length of the path described, during the revolution, by the centre of gravity of the perimeter.

2. Under the same circumstances, the volume of the solid generated is equal to the product of the area of the generating curve into the path described by the centre of gravity of the revolving area.

If A = the area of any plane curve,
P = the perimeter of the curve,
Y = the radius of the path described by the centre of gravity of the perimeter,
Z = ditto of area,
then the two theorems may be stated briefly,

(1) $2\pi YP$ = surface of the solid figure,
(2) $2\pi ZA$ = volume of the solid figure,

generated by revolving the curve around its axis.

As stated above, were the exact form of the cell known in any given species, we could by applying equation (1) derive its area with great accuracy, in spite of its bulgings and indentations. In the absence of this knowledge recourse must be had to equation (2). Since the volume of the cells is known, we can equate the expression $2\pi ZA$ to a definite quantity. But many curves still satisfy this condition. Fortunately we are able to limit the number of possibilities by making each conform to certain restrictions. Thus the diameter of the cell limits its maximum distance from the axis of rotation. Thickness is more difficult to fix. This value changes constantly as one progresses from the centre to the periphery. However, the maximum thickness can be arrived at in the following way.

Oxalated blood is placed on the rulings of a hæmocytometer. The cells are allowed to go into rouleaux. The lengths of several groups are measured and the total number of cells involved counted. The average maximum thickness of the cells can then be calculated. The results so obtained are: cat, $2{\cdot}1\mu$; rabbit, $2{\cdot}15\mu$; dog, $1{\cdot}95\mu$; man, $2{\cdot}05\mu$. As explained before, this method is inapplicable to goat's blood, the cells of which refuse to form rouleaux.

Meridional cross-sections of cells were drawn to a scale of 10,000 times the actual size, *i.e.* with 1·0 cm. representing 1·0 μ (Fig. 2). They

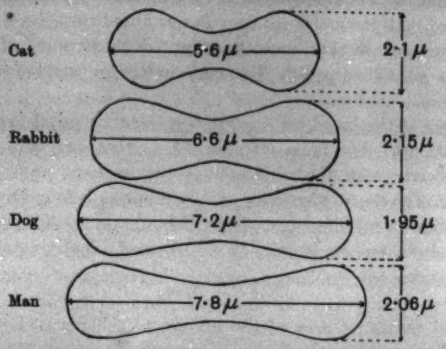

Fig. 2. Meridional cross-sections of the red blood cells of cat, rabbit, dog and man, drawn to the same scale, the outlines being derived with the help of Guldin's theorems.

were made just to fit within a rectangular figure whose length and breadth represented the maximum cell diameter and the maximum thickness respectively. To calculate the volume of the solid figure represented by this meridional cross-section, half the complete figure (symmetrical about its short axis) was cut out of stout card. Its area was determined in two ways: first, by drawing the curve on fine graph paper and estimating the area of the squares and fractions thereof enclosed, and secondly, by weighing the excised portion of card and comparing it with the weight of a known area of similar card. The two methods checked very well. The centre of gravity of the area was located by balancing the segment on a knife edge lying parallel to the axis. The other factor, Z, was obtained by measuring the distance between the axis and the centre of gravity, thus completing the left-hand side of the equation,

$$2\pi ZA = \text{Volume}.$$

The curve, still within the limits of the external rectangular figure, was then altered slightly until the volume by calculation was the same as the experimental volume of the cell. Thus a figure was obtained which conformed in diameter, volume and maximum thickness as well as in general configuration to a blood cell when enlarged 10,000 times. It will be noticed that the curves have to conform to all and each of the

restrictions laid down, viz. the experimentally measured diameter and maximum thickness, as well as the appearance of a blood cell, and must calculate out to the same volume (measured experimentally) as the blood cell of that particular species. It was found that there was practically only one curve that could be accepted unless some of the above restrictions were disregarded. The same procedure was followed for the red cell of each species.

The form of the blood cell having been established, it is a relatively simple matter to determine its area. A soft copper wire is bent to coincide exactly with the perimeter of the segment used in the last experiment. The centre of gravity of the perimeter is found by balancing the bent wire on a knife edge. Thus Y is obtained. The wire is then straightened out and its length, P, is measured. Inserting these values in the equation,

$$2\pi YP = \text{Area},$$

we can find the exact area of the individual cell for each species (see Table I).

These values for area do not agree with some previous estimations which have found their way into text-books. Some of these commonly quoted estimates are, however, on the face of them absurd. The geometrical figure which has minimal area for a given volume is the sphere. If now an author arrives at estimates of area which are equal to and sometimes less than that of a sphere of similar volume, this method of calculation is obviously erroneous. This criticism applies to certain of Bürker's estimates of area, as quoted by Ponder(13) and by Lovatt Evans(17). In the case of the corpuscles of man and of dog, in spite of their known discoid form, the area as given by Bürker is just equal to that of a sphere of similar volume. When Bürker comes to calculate the area of cells of progressively diminishing diameter, viz. rabbit, cat and goat (in this order), the original error becomes more and more accentuated, until in the case of the last-named animal he arrives at a computation of area which is only 60 p.c. of that of the sphere of equivalent volume.

On his figures Bürker based the conclusion that the hæmoglobin content of one cell divided by its surface area is constant. As we shall shortly see, his conclusion is probably correct, even though the data on which the conclusion is based are erroneous.

Ponder(13) improves slightly on Bürker's formula by regarding the cell as an ellipsoid of rotation and deducting a secondary ellipsoid representing the two concavities from the former. But he too calculates

the cells of cat and goat as having an area less than their respective spheres of similar volume, which simple error might have been avoided had his calculations been varied with occasional experimental determinations of volume.

In a cu. mm. of blood the total cell area, A, is given by the product of the individual cell area, a, by the blood count, N. We have seen that the individual cell area for the four species, man, dog, rabbit and cat, progressively decreases. On the other hand, the blood count, as we proceed from man to cat, steadily increases. It is of no little interest to note that in each case the blood count varies inversely as the individual cell area, the result being that the total cell area of all four species is practically constant, the figures in question being 7·24, 7·55, 7·52 and 7·35 sq. cm. respectively. As already stated, Bürker, on the basis of dubious data, arrived at an analogous inference, from which he deduced that the hæmoglobin of the red blood corpuscles probably has a surface distribution. The present results give added confirmation to Bürker's deduction.

A further relationship arises when one plots the volume of the cells of different species against their respective surface area. If we turn our attention for a moment to the ideal or theoretical cylindrical cell

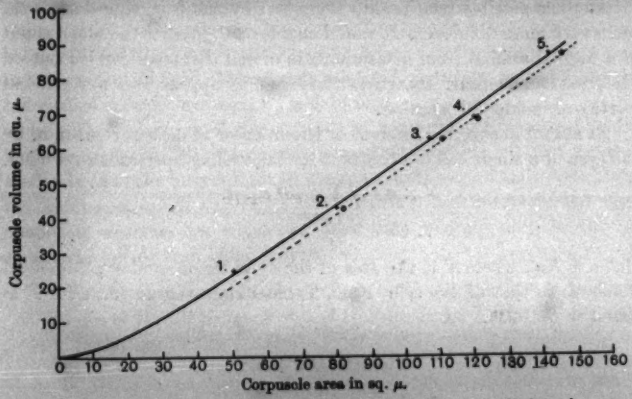

Fig. 3. The continuous line represents the volume-area relations of cylinders of constant height (1·8 μ). The crosses marked 1 to 5 represent the same relation for the cells of goat, cat, rabbit, dog and man calculated as cylinders. The encircled points 2 to 5 represent the area of the cells of cat, rabbit, dog and man when the area is calculated by Guldin's theorems.

of constant height (1·8μ), and plot a curve representing the relationship between its volume and its area, we obtain a graph such as is shown in Fig. 3. Knowing the volume and the diameter of the cells, one can calculate the dimensions of a cylinder of similar (theoretical) volume and diameter, and hence the area. This may be accomplished from the formula

$$A = (\pi D^2/2) + (4V/D).$$

When this is done, the points obtained (represented as crosses in Fig. 3) are found to lie surprisingly close to the curve for cylinders of constant height.

If on the same graph we plot the volume of the cell against the actual area as arrived at by Guldin's theorems, the points (enclosed in circles) form a second curve paralleling that for the hypothetical cylindrical cells. The relationship of the latter curve to the former indicates a slightly greater area for the actual as compared with the theoretical cell. This is only what one might expect from the curved configuration of the actual cells. It will be noted that the cells again appear to bear a fixed relationship to cylinders of similar volume and diameter. They are not mere haphazard figures but rather specialised forms of cylinders whose one common feature is that of constant height.

Another possible relationship suggested by the foregoing data is set forth with more diffidence. It would enable us to predict the blood count of a normal animal from measurements of cell diameter carried out on the dried blood smear. Bizarre as the idea may appear, it is nevertheless worthy of incidental mention.

As stated above, the product of the number of cells per cu. mm. by the area of a single cell is a constant for the various species here studied.

$$N \times a = 7{\cdot}4 \times 10^8 \text{ sq. } \mu.$$

$$\therefore\ N = \frac{7{\cdot}4 \times 10^8}{a}.$$

But $a = Ka'$, where a' is the area of the cylinder of similar volume and diameter to that of the cell. From Table I the average value for K is found to be 1·030.

$$a' = (2\pi D^2/4) + (2\pi Dh/2)$$

$$= \pi D\,(D/2 + h);$$

$$\therefore\ N = \frac{7{\cdot}4 \times 10^8}{\pi D/2\,(D+2h)\,K}.$$

Now, h is constant and equal to 1·8;

$$\therefore\ N = \frac{4{\cdot}56967 \times 10^8}{D\,(D+3{\cdot}6)}.$$

The formula, if applied to tables of cell diameter such as are given by Gulliver(14), might well enable us to form some conception of what the blood counts in many of these rare animals might be. For instance, *Tragulus javanicus*, listed as having a cell diameter of 2·0μ, would according to this rule have a cell count in the neighbourhood of 40 million per cu. mm. As a check on this hypothesis it would be interesting to ascertain the actual number of cells per cu. mm. in this animal's fresh blood. A practical use of such a formula, if substantiated, might be to indicate the degree of intrinsic anæmia over and above what has been compensated for by enlargement of the cells in the pathological condition recognised to be of the pernicious type. Prognosis might be aided by such knowledge.

Summary.

1. The thickness of the red blood cells of different species of mammals is found to be nearly the same in all cases. As a result any variation in volume of the cells of different mammals depends upon a variation merely of the average cell diameter.

2. The pathologically enlarged cells that occur in pernicious anæmia seem to undergo no increase in thickness. Hence simple eriometer measurements of increased diameter may be substituted for the more complicated measurements of increased corpuscle volume in diagnosis of this disease.

3. A new method, depending upon Guldin's theorems, for calculating the exact area of erythrocytes is described.

4. The total area of the red cells in 1 cu. mm. of blood is found to be the same for all the species investigated. This in turn tends to indicate that the hæmoglobin of the red cells is superficially distributed.

5. A method is suggested of estimating approximately the blood count in animals for which we have data relating only to the size (diameter) of the red blood corpuscles.

I wish to express my thanks to Prof. L. V. King of the Department of Physics of McGill University, and to Prof. John Tait of this Department, for advice in this research.

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THE INFLUENCE OF THE VAGUS ON THE ISLETS OF LANGERHANS. Part III. Further experiments on vagotomy.

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Evidence that the vagus may play a part in the control of blood-sugar level has been brought forward by many investigators (1), the majority of whom have suggested that this nerve, on stimulation, produces a secretion of insulin. Because of the grave disturbances of blood-sugar produced by abdominal operations, however, definite proof that the seat of action of the vagus in producing hypoglycæmia is in the islets of Langerhans is lacking, although Britton (2) claims to have shown that the pancreas is the organ primarily involved. Taken as a whole, the available evidence is strongly presumptive that the vagus contains secretory fibres to the islets of Langerhans. It has been shown, however, that for some weeks after section of the vagus in rabbits the sugar tolerance is not diminished but increased (3), indicating that the normal production of insulin is not only independent of vagus secretory impulses but may actually be held in check by tonic inhibitory action of the vagus and that after section of the nerve, since these inhibitory impulses no longer reach the islets, these latter now respond more readily to the appropriate stimulus for secretion. It is possible, however, that the phenomenon of increased sugar tolerance after vagus section is analogous to the increased susceptibility of the pupil to adrenaline which Byrne (4) has shown to occur even after section of the preganglionic sympathetic nerve supply; and that just as in the iris the muscle fibres when denervated respond more readily to adrenaline, so too the islets of Langerhans, after vagus section, respond more readily to whatever substance stimulates insulin secretion (? glucose).

If this is the true explanation of the result of vagotomy then this effect should not come on at once but only after the lapse of one or more days. If on the other hand there are fibres in the vagus that exert tonic inhibitory control over the islets, and if at the time when the nerve is cut this inhibitory control is in operation, not itself inhibited, then larger amounts of insulin should at once be liberated and the blood-sugar should fall as an immediate result of the section.

Whether at the time of the experiment such inhibitory influences should be in operation or not can only be determined in terms of the hypothesis itself. The hypothesis is that the normal mean level of sugar concentration in the blood is maintained in part by tonic inhibition of the islets, more pronounced when the blood-sugar is low, itself inhibited when this is raised. It is, therefore, by hypothesis, only when the blood-sugar is not raised above the mean level that section of the vagus would be likely to lower the level at once. Britton(3) found no fall on cutting the vagus; but in his experiments there was generally a high degree of hyperglycæmia.

But since, in addition to the influence of insulin, the carbohydrate store of the liver is concerned in the normal regulation of the concentration of sugar in the blood, a low level of blood-sugar may be corrected by a discharge of sugar from the glycogen store in the liver, and such a correction would mask any effect due to abolishing inhibitory action of the vagus on the islets by cutting this nerve. In order therefore to bring this latter effect into evidence, if it exists, it is desirable that (1) the sugar in the blood should not be above the mean level and also (2) that the chance of intervention of the liver should be diminished as far as possible. The conditions therefore under which it might be possible to detect by vagotomy experiments the existence of tonic inhibition of the islets by the vagus are defined, though their control may not be always equally attainable. Cats under amytal anæsthesia were used, the amytal being given subcutaneously under ether and the animals left for two hours or more so that hyperglycæmia might subside. The necessary preliminary operative work was then done and a further period allowed to elapse during which blood-sugar estimations were made at half-hourly intervals till the sugar was constant. The vagus was then cut and sugar estimations continued.

In the first series, the right vagus was cut in the neck, the nerve having been carefully exposed and looped with thread in a preliminary operation. It will be seen from Table I that in all but two cases (Expts. 5 and 6) section of the nerve was followed by a definite fall in blood-sugar. In one of the apparent exceptions there is a suspicion of a fall, while in both of them the sugar level shows a rise beginning a half and one hour respectively after cutting the vagus. It is possible to explain this hyperglycæmia as due to liberation of liver glycogen in excess of that necessary to counteract the tendency to hypoglycæmia produced by vagus section; it is moreover to be observed that in both cases the initial sugar level is high above the normal mean level. A similar

phenomenon is seen in Exp. 1, where, however, a definite fall precedes the rise.

TABLE I.

Exp.	Before section				After section					
Hrs.	1½	1	½	0	½	1	1½	2	2½	3
1.	·110	—	·114	·112	·094	·083	·123	·105	·080	—
2.	·134	·128	·132	·129	·111	·100	·088	·090	·089	·092
3.	·091	·090	·090	·090	·073	·066	·063	·070	—	—
4.	·107	·102	·103	·101	·082	·090	·068	·062	·070	—
5.	·142	·145	·141	·147	·149	·169	·130	—	—	—
6.	—	·132	·136	·134	·129	·130	·157	·160	·145	·137

It may be suggested that the lowering of blood-sugar is not the result of cutting the nerve but of stimulation in the act of cutting. To meet this criticism, in Exp. 1 the nerve was frozen with solid carbon dioxide before section and in Exp. 4 conduction in the nerve distal to the cut was blocked by a constant current. Moreover, it has been shown by Britton that long periods of electrical stimulation are necessary to produce hypoglycæmia, which makes it improbable that the momentary stimulus of cutting with sharp scissors could be responsible for the results given above.

In the attempt to localise the organ on which the vagus acts, the first step was to determine the effect of cutting the right vagus as it enters the abdominal cavity on the posterior aspect of the œsophagus. The nerve was exposed with as little interference with other parts as possible, looped with thread and the abdominal wound temporarily closed by clips; blood-sugar estimations were then made till a steady level was maintained. Then the wound was reopened and the nerve cut by a pair of sharp long-bladed scissors. It will be seen from Table II that here again vagus section was followed by a fall in blood-sugar.

TABLE II.

Exp.	Before section			After section				
Hrs.	1	½	0	½	1	1½	2	2½
7.	·136	·138	·135	·122	·106	·090	·100	·104
8.	·120	·125	·127	·123	·115	·127	·136	·120
9.	·123	·120	·124	·110	·105	·138	·143	—

It is apparent that interference with pulmonary or cardiac function is not responsible for the lowering of sugar level after cutting the vagus.

The phenomenon might be due to cutting off impulses normally passing to the liver or suprarenals, but in two experiments neither of these factors appeared to play a part. In one, both suprarenals were eliminated by incising the peritoneum, raising the glands and tying a ligature round the pedicle. In the second case, in addition to tying off the

suprarenals, all nervous connections of the liver in the hepatic pedicle were severed. The right vagus was cut in the neck as previously described. In both cases the result was similar to those in Table I.

TABLE III.

Exp.	Before section				After section					
Hrs.	1½	1	½	0	½	1	1½	2	2½	3
10.	·116	·117	·120	·116	·090	·078	·090	·075	·102	·090
11.	·102	·106	·105	·109	·083	·078	·079	·074	·084	·077

Several experiments in which an attempt to eliminate the pancreas or the duodenum from the circulation prior to cutting the vagus have failed because of the abnormally wide fluctuations of blood-sugar resulting from the operative interference. Dixon(5) has shown that extracts of duodenal mucous membrane will lower blood-sugar when injected into dogs, but there is no evidence to show that the duodenum plays any part in the control of blood-sugar, despite Pflüger's attempts to demonstrate its relation to diabetes(6). Nor is it known that any other part of the alimentary canal influences the sugar content of the blood in any way other than passively during absorption. In the present state of knowledge, therefore, it may be presumed that the pancreas is the organ responsible for the phenomenon.

SUMMARY.

Section of the right vagus above or below the diaphragm in cats under amytal anæsthesia produces an immediate fall in blood-sugar, the degree of which varies with (*a*) the initial blood-sugar level and (*b*) the glycogen reserve. It is suggested that this effect is produced by cutting off tonic inhibitory impulses which control insulin secretion.

I wish to express my thanks to Professor Leathes for his kindly interest and valuable criticism.

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STUDIES ON THE INTERNAL SECRETIONS OF THE OVARY. V. The œstrus-inhibiting function of the corpus luteum[1].

By A. S. PARKES (*Beit Memorial Research Fellow*) and C. W. BELLERBY.

(*From the Department of Physiology and Biochemistry, University College, London.*)

I. Introduction.

Experiments have made it clear that the corpus luteum is involved in (*a*) the development of the uterus, preparatory to the reception of the fertilised ovum, (*b*) the inhibition of œstrus and ovulation during pregnancy, (*c*) the maintenance of pregnancy, and (*d*) the development of the mammary glands. Attempts to prepare extracts which will produce such effects in the absence of corpora lutea, however, have met with but little success, except possibly as regards the inhibition of œstrus.

The concept of an action inhibiting ovulation was originally put forward by Beard(1) and by Prenant(2) and was based on the general functional correlation which exists between the development of the corpus luteum and the absence of œstrus (except during anœstrus). During recent years this hypothesis has been amplified in many ways, and the chief evidence may be summarised as follows:

(*a*) During pregnancy (in probably all mammals), during lactation (in the mouse and the rat) and during pseudo-pregnancy (in the rabbit and ferret), the development of the corpora lutea is associated with the absence of œstrus and ovulation.

(*b*) In the cow (Hammond(3)), and in the guinea-pig (Loeb(4), and Papanicolaou(5)) the removal of the corpora lutea of ovulation expedites the appearance of the next œstrous period. This effect is apparently limited to animals which have a marked luteal phase in the cycle. In the unmated mouse the elimination of the corpora lutea of ovulation by X-ray sterilisation was not found to affect the length of cycle. The unmated mouse, however, has no luteal phase to eliminate, whereas the luteal phase in the guinea-pig is very prominent.

[1] Nos. I to IV of this series of studies appeared in Vols. 61 and 62 of this Journal.

(*c*) Abnormal persistence of corpora lutea coincides with cessation of the œstrous cycle. Thus the human menstrual cycle may cease as a result of the persistence of corpora lutea, and the removal of these is followed by recurrence of the cycle (Ochsnier(6)). So too in the cow (Hess(7), Williams(8)), expulsion of the persistent corpora lutea by manipulation *per rectum* is a well recognised treatment for sterility.

(*d*) Experimental prolongation of the functional life of the corpora lutea, as by hysterectomy in the guinea-pig (Loeb(9)), results in the suppression of œstrus.

Attempts to obtain this effect in the normal animal by injection of corpus luteum extracts have not proved very successful. Corner and Hurni(10) report negative results with normal rats, while Loeb(11) failed to obtain regular positive results on the guinea-pig. Pearl and Surface(12) appear to have obtained some success in stopping hens from laying by injecting water extracts of a commercial preparation of dried corpus luteum. Subsequently Kennedy(13) reported positive results on the rabbit: saline extracts of dried (commercial) corpus luteum were injected some time before mating, with the result that ovulation was suppressed. Recently Papanicolaou(14) has described inhibition of œstrus in the guinea-pig by injection of extracts of corpus luteum. Johnston and Gould(15), however, failed to inhibit the action of œstrus-producing extracts of the ovary by the simultaneous injection of extracts of corpus luteum prepared in the same way.

When all the data, including those of functional correlation, are considered, it appears that the corpora lutea of ovulation in some animals, and the (normally or abnormally) persistent corpora lutea of apparently all mammals, have an œstrus inhibiting action.

In view of this conclusion, and considering the general improbability that œstrin is the sole regulator of the female sexual cycle, the persistent reports that extracts which produce œstrus can be prepared from corpora lutea have become increasingly anomalous.

Iscovesco(16), Seitz, Wintz and Fingerhut(17), Okinschitz(18), Herrmann(19), Frank and Gustavson(20) have all claimed to have obtained from corpora lutea extracts that produce œstrus. Allen and Doisy(21) have failed to do so with material from the cow and the pig, but have found activity in preparations of human corpora lutea. Quite recently Glimm and Wadehn(22) have also reported œstrus-producing activity in extracts of corpus luteum. Johnston and Gould(15), however, failed to obtain œstrus producing extracts of pig corpora lutea.

Summing up the evidence it may be said that there is good reason

to suppose that the corpus luteum has, among others, the function of inhibiting œstrus, but several workers, on the other hand, have made from corpora lutea extracts that produce œstrus. The work described in the present paper relieves to some extent this paradoxical situation, (*a*) by showing that œstrin, if present at all, is not elaborated by the corpus luteum, and (*b*) by confirming previous workers who have produced extracts of corpora lutea that inhibit œstrus.

II. Methods and materials.

Detection of œstrus. In both normal and injected ovariotomised mice the detection of œstrus has been made by the vaginal smear method (Allen (23) as discussed before (24)).

Extraction and testing of the œstrus hormone. The testing of corpora lutea for œstrin consisted essentially of the extraction of hollow specimens, the aim being to examine the fluid content separately from the tissue "shell." The ovaries (cow) were received frozen and the corpora lutea were squeezed out before thawing was complete; they were then slit open, and solid specimens were washed and minced without further treatment. The fluid centre of hollow corpora, while still frozen, was removed for separate extraction; as an extra precaution the "shells" were carefully washed before extraction. Hollow corpora lutea are not plentiful, and the amount of fluid obtained in each batch was relatively small.

The extraction of the fluid for the present purpose was carried out as previously described (25) for liquor folliculi, while the "shells" and the solid corpora were treated in the same way as ovarian tissue.

All these extracts were tested for œstrin on mice in the manner previously described (25), and the following values were ascertained or calculated:

M.U. Mouse unit. The least amount of an extract given at one time required to produce œstrous symptoms in an ovariotomised mouse.

M.U.K. (L.). Mouse units per kilogram (litre) of original tissue.

Preparation and testing of œstrus-inhibiting extracts. The methods employed in the endeavour to obtain extracts capable of inhibiting œstrus are described later. As regards the testing of extracts for œstrus-inhibiting properties two possibilities suggested themselves: (*a*) to inject normal animals of known œstrous history and ascertain if the cycle was interfered with, and (*b*) to inject into ovariotomised animals a mixture of 1 M.U. of œstrin and the extract to be tested, *i.e.* to ascertain

if the extract would inhibit the action of œstrin. The former was much more simple and was used as a routine. If œstrus was inhibited so long as injection was continued (in most cases the inhibition continued for some time after the last injection) the extract was considered to be active.

III. Histology of the corpus luteum.

Many workers have maintained that the luteal cells are entirely derived from the membrana granulosa, others that they are formed from the theca interna cells, and some that both contribute to their formation. Corner(36) in the sow, McNutt(27) in the cow, Hill and Gatenby(28) in *Platypus*, and Gatenby(29) in the human have all arrived at this last conclusion. It has been found by Dr Brambell that this is also true of the mouse.

There has also been considerable diversity of opinion concerning the nature of the central cavity filled with fluid found in some corpora lutea. Some workers have considered these to be cystic. Allen(23), however, finds that in the mouse the corpus luteum forming in the ruptured follicle becomes redistended with tertiary liquor folliculi a few hours after ovulation. This central cavity is then gradually reduced and finally filled up by the growth of the luteal tissue. Hammond(3) arrives at a similar conclusion in respect of the cow. He states that the newly formed corpus luteum contains a central blood clot. The central cavity then becomes filled with a glairy albuminous fluid similar to the liquor folliculi, the blood clot being still apparent at the point of rupture. McNutt(27), however, arrives at an opposite conclusion, also for the cow, "A redistention of the central cavity may or may not take place. When it does take place to any appreciable extent it results in a small cyst being formed which remains here throughout the life of the corpus luteum without apparently interfering with its normal cycle."

In the mouse a central fluid-filled cavity normally occurs in the newly-formed corpus luteum and later, in the majority of cases, disappears. Occasionally a true cyst is formed which persists indefinitely. However this may be there can be little doubt that the fluid content is the result of the formation of liquor folliculi, continued after the rupture of the mature follicle.

IV. The distribution of œstrin in the corpus luteum.

Our results confirmed previous workers in showing that a slight œstrus-producing activity could be detected in unsorted batches of

corpora lutea, though this was sufficiently small to suggest that it might be due to the fluid of the hollow specimens.

Extracts were therefore made of solid corpora lutea and Table I summarises the results of these extractions.

TABLE I.

Extract No.	Nature of tissue	Amount of tissue grm.	Mouse units	M.U.K.
6	Whole unsorted corpora lutea	150	2	13
7	—	220	4	18
8	Solid corpora lutea	960	No œstrin detected	
9	—	930	"	
10	—	100	"	
11	—	145	"	

The solid corpora lutea, therefore, do not on extraction yield detectable amounts of œstrin. In the case of Extract No. 9, the residual tissue of the ovaries with the large follicles punctured, amounting to 1050 grm. gave a yield of 253 M.U. or 241 M.U.K.

Next, in four batches of hollow corpora lutea the tissue and fluid were extracted separately with the following results:

TABLE II. Extraction of tissue and fluid of hollow corpora lutea.

Extract of	Wt. in grm. or vol. in c.c.	Extract yield grm.	M.U. of whole yield	M.U.L. or M.U.K.
Solid 1	200	1·8	0	0
Fluid 1	160	0·27	24	149
Solid 2	340	3·5	0	0
Fluid 2	95	0·09	15	158
Solid 3	125	1·2	0	0
Fluid 3	33	0·13	10	303
Solid 4	65	1·5	8	123
Fluid 4	10	0·05	6	600

In only one extract of tissue was œstrus-producing activity detected, and in this case it was comparatively slight. It would seem, therefore, that œstrin is not usually present in the tissue of hollow corpora lutea, and since it was absent from solid corpora lutea, there is some reason to suppose that the tissue of neither type contains œstrin, and hence, presumably, does not elaborate it.

Œstrin was, however, found in appreciable amounts in the fluid from hollow corpora lutea, which suggests that the positive results obtained by many workers for corpora lutea apparently depend on the presence of the hollow bodies containing œstrin in their central fluid.

V. Preparation and action of the œstrus-inhibiting extract.

Preliminary attempts to prepare œstrus-inhibiting extracts were not successful; among these were extractions by the method described in Part I for œstrus-producing extracts; extraction with water, and extraction with alcohol, followed by acetone and then by ether.

Indications of success were obtained by the use of press-juice from the fresh corpora lutea, but the preparation was unsatisfactory owing to the local reactions which attended the use of the untreated juice, and a further complication arose from the fact that the juice, even if kept in cold storage, soon lost such potency as it had at first possessed.

Preliminary fractionations of the fluid, avoiding heat and exposure to the air, indicated that the labile active principle was soluble in acetone and in ether. An attempt was next made to dry and extract the fresh tissue with acetone, evaporate the acetone at 40° *in vacuo*, and take up the residue with ether, but the process, though partially successful was abandoned owing to technical difficulties. Fractional precipitation of the acetone extract with water was also unsuccessful, as was a similar treatment of the acetone extract of tissue dried by Na_2SO_4.

The method of preparation finally arrived at was as follows. Solid corpora lutea (usually about 200 grm.) were minced and ground up with anhydrous Na_2SO_4, and then allowed to stand for not more than 24 hours in ether. The extract was then filtered and evaporated down *in vacuo* and the residue extracted with acetone, the acetone solution evaporated down *in vacuo*, and the oil obtained either injected as such or emulsified in ·5 p.c. $NaHCO_3$. The fraction insoluble in acetone was inactive. Originally, the whole ether extract was taken down at once and the emulsified product kept in cold store, but better results were obtained by preparing fresh oil each day from a portion of the ether solution, though even the ether extract rapidly loses its activity. This process differs but little in principle from many previously tried, except in the precautions taken to have the extract made as soon as possible from fresh material and to avoid exposure to heat and air. The only extract made by this method which showed doubtful activity was one from ovaries kept in cold store for three days. The other eleven extracts all gave positive results, the amount of oil injected per day being usually ·2 c.c. Less than this amount was ineffective. A diagrammatic summary of the behaviour of animals injected with oil produced by this process is given in Fig. 1.

Relative to this diagram the following remarks may be made:

(a) Eleven of the twelve extracts made gave definite inhibition of œstrus. Four of the six tests of Ex. 37 (the doubtfully active extract

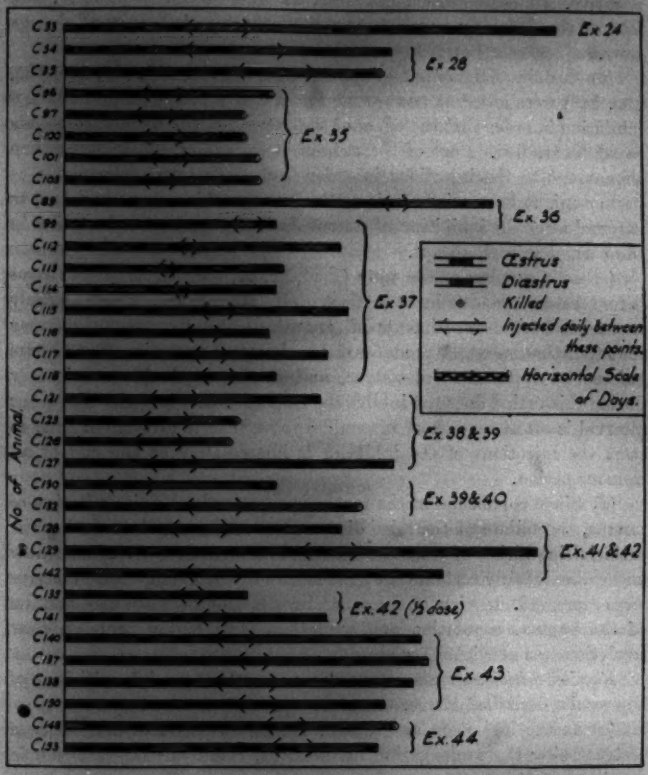

Fig. 1. Œstrous history of animals injected with œstrus-inhibiting extracts of corpora lutea.

mentioned above) gave negative results, but in two of the animals in question injection was made for only two days. Two of the tests with Ex. 42 also gave no results, but the previous injections make it clear

that this extract was active, and the two animals (*C* 135 and *C* 141) returning negative results had a reduced dose for a shorter period than any of the others. One of the tests of Ex. 36 (on *C* 99) was doubtfully negative. Of the 34 tests, therefore, 27 were definitely positive, and four of the definitely negative ones were short period or small dosage tests.

(*b*) Certain of the animals (*e.g.* *C* 35, 96, 97, 100, 101, 103, 123, 126, 132, 148) were killed at or towards the end of a considerable period of inhibition in order that the effect of the extract on the mammary glands could be studied. Most of the animals were examined for some time subsequent to the injections in order that the return of the cycle to normal might be studied. This return to normal is not gradual but, as is found after the inhibition of œstrus during pregnancy or lactation in the normal animal, abrupt.

(*c*) In a number of the tests (*C* 34, 35, 97, etc.) injection 1–2 days before œstrus was due had no effect on its appearance. This is readily explained by the experiments of Brambell and Parkes(30) showing that the stimulus which produces œstrus becomes operative 36–48 hours before the appearance of œstrus, and that even double ovariotomy during this period does not inhibit the next œstrus. Since the dioestrous interval is often very short it would appear that the optimum time to start the injections of the inhibitor is immediately at the end of an œstrous period.

(*d*) Since copulation in the mouse is a perfectly definite criterion of œstrus, the failure of the mice discussed above to copulate during the artificial inhibition of œstrus confirms the diagnosis by vaginal smear and makes it fairly certain that all the extra-ovarian symptoms of œstrus were repressed. In view of the general correlation between cornification of the vagina, copulation and ovulation, it is highly probable that ovulation was also inhibited.

Possible criticisms of the results. There are three possible criticisms of the results described above.

(*a*) Is the inhibition a real one? *i.e.* the criterion of inhibition of œstrus being the prolongation of dioestrus, could normal variation in length account for the results?

(*b*) What causes the variation in the degree of inhibition found?

(*c*) If the inhibition is real, is it due to the large injections of lipoid material or to toxicity of the extracts rather than to a physiological œstrus-inhibiting substance?

Since the animals used for testing were chosen because of their

regular œstrous history, the explanation of the results on a chance basis would involve a degree of coincidence out of all probability. Further, in very many of these animals showing inhibition after injection the vaginal smears changed from the milky type of diœstrus to the thick viscous one of pregnancy or pseudo-pregnancy.

The variation in the degree of inhibition obtained is great, but since no attempt has yet been made to assay the extracts, their relative potency was unknown. Moreover, the nature of the material injected must have made absorption very uneven.

The doses of total material administered to produce inhibition are, of course, relatively enormous, but since a large number of injections of similar material produced no inhibition it is quite clear that the effect is not due to injecting large amounts of emulsion or to toxicity of the extracts. Fig. 1 represents all the tests of extracts of corpora lutea made by this method, except those on three animals which died prematurely during an epidemic, and it is reasonable, therefore, to conclude that these extracts exhibited definite, though variable, œstrus-inhibiting properties. Extracts of whole ovary less corpora lutea, made and tested in the same manner, gave no such results; their effect was, as would be expected, rather to promote œstrus, and it would appear that the inhibiting substance is confined to the corpus luteum.

VI. Discussion.

(*a*) *The distribution of œstrin in the corpus luteum.*

It is shown elsewhere[30] that the œstrus-producing stimulus becomes operative (about 1½–2 days after the end of the previous œstrus) at about the time when the corpus luteum of ovulation reaches its maximum histological development. At present, it appears to be difficult to attach any definite meaning to this correlation. The corpus luteum is certainly not the essential organ of œstrin elaboration. The time of maximum histological development of the corpus luteum is not necessarily that of greatest functional activity, and there is definite evidence that the corpus luteum of ovulation in the unmated cycle has essentially neither an œstrus-promoting nor an œstrus-inhibiting function. The elimination of the corpora lutea as cyclic structures in the sterilised female, for instance, does not affect the periodicity of œstrus. The apparent discrepancy with the experiments of Loeb on the guinea-pig[4] and Hammond on the cow[3], where removal of the corpora lutea expedited the next œstrus, is due to the prominence of the luteal phase

in the cycle of the guinea-pig and the cow, as compared with its absence in the mouse; the corpus luteum of ovulation appears to be functionless in the mouse unless caused to become persistent by sterile copulation, pregnancy, or lactation.

In view of the correlation between persistence of corpora lutea and inhibition of œstrus, reports of the presence of œstrin in corpora lutea are at first sight surprising, but the results given in this paper tend to remove this anomaly. Nearly all workers have used cow material for extraction, and we have shown above that their œstrin content, in this species at any rate, is almost limited to the fluid in the centre of the hollow corpora lutea. This fluid, being probably homologous with liquor folliculi, may be supposed to obtain its œstrin from the same source, and the apparent absence of œstrin from solid corpus luteum seems to preclude the possibility of it being formed by luteal tissue.

(*b*) *The interaction of œstrus producer and œstrus inhibitor.*

The inference from these experiments, that the corpus luteum does not produce œstrin, but on the contrary, produces a substance inhibiting œstrus, supports the work of Papanicolaou(14) who claims to have inhibited œstrus in guinea-pigs by injection of extracts of corpus luteum. Since, however, this author gives no particulars of the preparation of the extracts, it is not possible to compare them with the one used in the present experiments. Our results strongly support the opinion put forward in Part IV of this series, that the œstrus-producing hormone cannot be the sole regulator of the female reproductive cycle. The view that at least two ovarian secretions are concerned in the regulating mechanism has been held for many years by Marshall(31) and by Loeb(32), among others, and has recently been maintained by Courrier(33). It appears that the position taken up by Frank and his co-workers, and also to a lesser degree by Allen, that œstrin is the controlling factor at all stages of the fertile cycle, and is produced successively by the Graafian follicle, the corpus luteum, and the placenta, is rapidly becoming untenable. It is extremely doubtful whether the œstrus-producing hormone, often erroneously called the "follicular hormone" (it is produced freely in the complete absence of follicles), is even the most important ovarian secretion, and it has certainly no claim to be considered as "the ovarian hormone."

The results described above imply an antagonistic action between the œstrus-promoting hormone and the œstrus-inhibiting action of

the persistent corpus luteum. This is in keeping with the previous work (34, 35) showing that the œstrus-inhibiting action of the persistent corpora lutea which normally overrides the œstrus-promoting stimulus during pregnancy and lactation can in turn be overridden by the injection of excessive amounts of œstrin[1]. We thus arrive at the conception of the alternating dominance, in the complete fertile cycle, of œstrus promoter and œstrus inhibitor, it being possible to override each in its naturally dominant phase by adequate augmentation of the other.

At present very little can be said about the method of action of the inhibitor substance of the corpus luteum, *i.e.* the manner in which it postpones the operation of the œstrus-producing hormone. The way in which one inhibits the other and the way in which the natural balance can be disturbed by injection of one or the other almost suggests some kind of chemical "neutralisation" between the œstrus-producing substance and the œstrus-inhibiting one. On the other hand there are facts which imply that other endocrine organs such as the pituitary (38, 39) enter into the mechanism of œstrus production and inhibition. One point can, however, be stated quite definitely. Whatever the exact mechanism of œstrus inhibition may be, it cannot be a local action of the corpora lutea on the rest of the ovary. This fact is shown quite adequately by animals which ovulate from one ovary only at each œstrus period (*e.g.* the cow); the corpus luteum in the one ovary is quite sufficient to inhibit the œstrus-producing activity of both ovaries. Experimental confirmation of this fact has been obtained by means of experiments which will be reported fully later.

As has been pointed out at the beginning of this paper, it is clear that the inhibition of œstrus is only one of the functions of the corpus luteum, and one function having been simulated to some extent by injection of extracts, the question immediately arises as to whether the same extract will perform the other functions. No attempt can be made to answer this question as yet, but some preliminary experiments have been made. Some of the animals represented in Fig. 1 were killed for the purpose of examining the mammary glands to see if any semblance of pregnancy changes had been produced. The changes in the glands during early pregnancy are, however, very indefinite, and it was decided that some such animal as the rabbit, showing definite mammary changes

[1] Loeb (36) failed to produce œstrus during pregnancy by injection of œstrin in the guinea-pig, but probably the amounts used were insufficient. Smith (37) obtained on the rat results identical with those reported on the mouse in Part II of this series (34).

during early pregnancy and pseudo-pregnancy, would have to be used for these experiments.

Standardisation of the œstrus-inhibiting substance. Papanicolaou[14] suggested that the œstrus inhibitor should be assayed by the length of time for which the next œstrous period was inhibited. Since, however, it is impossible to tell exactly at what time the next period of œstrus should have occurred, this method would have grave objections. It seems most probable that the power of inhibiting œstrus possessed by extracts of corpus luteum will have to be standardised against the power of a known amount of œstrin to produce œstrus, say one mouse unit. A mouse unit of the inhibitor would then be the amount required to neutralise the action of 1 M.U. of œstrin. It is clear, however, that much work, particularly in the direction of purification of the inhibitor and more accurate standardisation of œstrin, will have to be done before much headway is made in assaying the inhibitor.

VII. Summary.

(1) In corpora lutea of the cow œstrin is found only in the fluid contained in the cavities of hollow specimens, and since this fluid appears to be homologous with liquor folliculi it may be supposed to obtain its œstrin from the same source as does the latter. The absence of œstrin from solid corpora lutea seems to dispose of the suggestion that it is elaborated by the corpus luteum.

(2) Functional correlation suggests most strongly that, among other functions, the persistent corpus luteum of pregnancy (and lactation in mice and rats) inhibits œstrus. In this paper an account is given of the preparation of an extract of corpus lutuem which on subcutaneous injection inhibits œstrus in the normal unmated mouse.

(3) Possible criticisms of the results are discussed, together with their bearing on the general theory of the œstrous cycle and the relation of the œstrus-inhibiting extract to the other functions of the corpus luteum.

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QUANTITATIVE OBSERVATIONS ON THYROXINE AND ALLIED SUBSTANCES. I. The use of tadpoles.

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In 1912 Gudernatsch(1) discovered a remarkable effect of thyroid preparations on tadpoles. They shrink rapidly in size and their metamorphosis is hastened. The phenomenon has been described in great detail by Romeis(2), Kahn(3), and others. Various workers have suggested that thyroid preparations should be assayed by methods depending on these phenomena. Swingle(4) discusses these papers and criticises them on the ground that the results are not a specific effect of the active principle itself, but may be produced in varying degree by almost any iodine compound. In most of these experiments the tadpoles were given repeated doses of the substance to be tested throughout the period of the experiment. Romeis(5), however, has shown that if tadpoles are immersed for a short time in a dilute solution of thyroxine the characteristic effects develop several days later. He found that the concentration of diiodotyrosine necessary to produce the same effects by such a method was hundreds of times larger than that of thyroxine. It seemed probable that under these conditions the test would be more specific. If the tadpoles were almost continuously in the presence of high concentrations of iodine, their own thyroid glands might be expected to be maintained in a perpetual state of hyperactivity, but phenomena which occur some days after a short immersion in a toxic concentration would not be so readily produced by substances which had no specific connection with the active principle of the gland.

Dr C. R. Harington kindly offered me the opportunity of testing the biological effects of the synthetic and natural thyroxines. At the same time he submitted to me a number of intermediate and related compounds which he has prepared and identified. I took the opportunity in the early summer of this year to examine the effects of this interesting series on tadpoles. Unfortunately the work was still incomplete when tadpoles became no longer available. Pending the examination of these compounds by other biological methods, and the resumption of the tadpole

tests next spring, it appears worth while to put on record the results already obtained.

Methods.

There is some doubt as to the best criterion to take of the effect on the tadpoles. Romeis suggested that the results should depend on the date of the appearance of the hind legs or front legs. This is in many ways a convenient end-point, though the observation is not so simple as might be supposed. Every tadpole must be examined separately, as the leg buds are difficult to see on their first appearance. This examination is conveniently achieved by placing the tadpoles on a piece of looking-glass in a bright light. Other changes of a less definite nature, such as the change in shape of the body, will often suffice to place different substances in the order of their activities in producing metamorphosis. Lenhart (6) laid stress on the interval which elapsed before all the tadpoles were dead. Such a method could only be satisfactory if it were possible to exclude death from causes other than the specific effect of the substance under test.

It was thought probable that some more definitely quantitative measurement would give a more trustworthy indication of the effects of different doses. One of the most marked changes is a great diminution of the length of the gut, and measurements of this have been made by Swingle (7), but this method was not adopted because it was wished to follow the course of development of the effect, and a measurement of the length of the gut can only be made once on each tadpole.

The most obvious change which occurs is a rapid diminution in size. This appears to be due partly to the hastening of the normal processes of metamorphosis and partly to the wasting which is associated with the increase in metabolic rate. The simplest measurement to make, and the one most usually made, is that of the total length, but a measurement of volume or weight would, of course, show much larger differences, and might thus be thought likely to prove the most sensitive index of the effect.

The volume may be determined by drying the tadpoles and adding them to water in a measuring cylinder (2), but it is difficult to remove water completely from the surface without injuring them. If small tadpoles are strained on a coffee strainer the volume of the water which fills up the spaces between them may be almost equal to that of the tadpoles themselves. Volumes have, therefore, been determined less directly by taking 12 tadpoles and adding water to make a total volume of 2 c.c. in a small cylinder, and then adding 1 c.c. of a dilution of cochineal (liq.

cocci about one drop to 10 c.c.). The mixture is then shaken and filtered, and the filtrate compared with the original dilution in a colorimeter. A simple calculation gives the volume of water by which the 1 c.c. of cochineal solution has been diluted, and so, by difference from 2 c.c., the volume of the tadpoles. Various other pigments have been tried, but have been rejected, either because they were toxic (methylene blue) or because they were absorbed (Indian ink). Tadpoles were kept for one hour in diluted cochineal without producing any change in the depth or quality of its colour. They were also kept for 24 hours in the solution without showing immediate signs of toxic effects. The determination of volumes was, however, eventually abandoned owing to the fact that the body cavities of the tadpoles occasionally became distended with watery effusion. Though this distension did not appear to be caused by the short immersions in cochineal or by the iodine preparations, it obviously interfered with the accuracy of the measurement of volume as an index of growth or general shrinkage. The same objections would apply to the measurement of the weight of tadpoles, which was adopted by McCarrison(8) as an index of their growth.

It was, therefore, decided to measure the over-all length of each tadpole. They were sucked up one by one into a piece of glass tubing and the water was run out. When they became immobile in extension, they were held against a scale and measured to the nearest millimetre. Tadpoles taken from stock were thus measured and placed in four or six jars so that all the tadpoles in one jar were equal in length. A number of similar batches of twelve tadpoles was then prepared by taking in each batch an equal number of tadpoles from each jar. It is difficult to interpret the results when some of the tadpoles die during the period of observation and all such results have been neglected. The mean daily length of a batch of twelve controls showed steady growth as is shown in Figs. 1 and 2.

In order to obtain an idea of the errors introduced by individual variations among the tadpoles, the figures obtained for the lengths of the tadpoles in the same batch were arranged in order of size, and the amount that each tadpole had grown was calculated on the assumption that this procedure would arrange them in the same order at any stage of the experiment. It was found that the administration of thyroxine greatly increased the variability among the tadpoles, the small tadpoles showing larger absolute decreases in length than the large ones. The difference between the means of the lengths of two batches, however, one of which had received twice as much thyroxine as the other, was greater than

three times the standard deviation of the differences of the means for batches of twelve, as calculated by the mean squares method. The fact

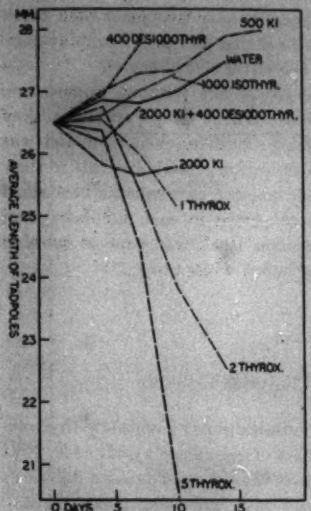


Fig. 1.

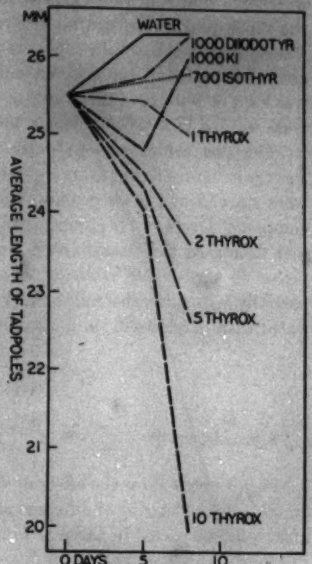


Fig. 2.

Fig. 1. The effect on the average length of batches of 12 tadpoles, hatched 65 days before, of an immersion for 24 hours in solutions whose concentrations are given in parts per ten millions. ISOTHYR represents a substance whose name and formula are given in the text which is isomeric with thyroxine. No measurements are recorded after the first tadpole in a batch died.

Fig. 2. The effect on the average length of batches of 12 tadpoles, hatched 59 days before, of an immersion for 24 hours in various solutions. The notation is as in Fig. 1. DIIODOTYR denotes a solution of 3, 5 diiodotyrosine.

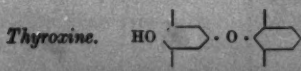
that a given batch of tadpoles shows a diminution of average length is of course not in itself sufficient evidence of occurrence of the phenomenon described by Gudernatsch, but in the presence of other evidence of the nature of the effect it gives a good indication of its magnitude.

Each batch of twelve tadpoles (*Rana temporaria*) was immersed for 24 or 48 hours in 500 c.c. of a clear solution, in tap-water, of one of the

compounds under test, a small quantity of sodium carbonate being added when necessary to secure complete solution. An experiment in which the tadpoles were exposed for a like period to carbonate alone in the same dilution showed no difference from the control with pure tap-water, so that a batch in tap-water was considered to give an adequate control in each experiment. After this preliminary treatment each batch was kept in 500 c.c. of tap-water in a two-pound jam-jar. Each day they were washed in a coffee-strainer, and the water was changed.

The diet which the tadpoles receive is not without importance. Jarisch (9) found that tadpoles were most sensitive to thyroid when they were starved or fed on protein, and least sensitive when their diet was complete. Lenhart (6) found that the addition of carbohydrate to their diet decreased their sensitivity. In the present experiments two small pieces of hard-boiled white of egg were placed in each jar daily, and partially eaten by the tadpoles. The main stock was kept in running water with pond-weeds, and also given boiled white of egg.

Results.

Thyroxine. 

Figs. 1 and 2 show the effect of various dilutions of synthetic thyroxin on the average lengths of different batches of tadpoles. It will be seen that after a latent period of three or four days they showed definite decreases in length, and that the effect increased with increasing concentration. Fig. 3 shows the result of a comparison of natural thyroxine with synthetic thyroxine, both in the racemic condition. The natural thyroxine appears in this test to have an equal potency with the synthetic thyroxine. The counting of legs gave similar but less definite results.

Potassium iodide. K|.

It will be seen in Figs. 1 and 2 that high concentrations of potassium iodide produced a smaller effect on the length, which developed and passed away more rapidly. There was no other evidence of changes in the tadpoles such as are produced by thyroxine.

Swingle (7) has produced the characteristic thyroid effect on normal tadpoles and on tadpoles whose development had been arrested by thyroidectomy by mixing various inorganic iodine preparations with their food. In these experiments, however, potassium iodide differed from the other substances tested in that it increased the rate of growth of the

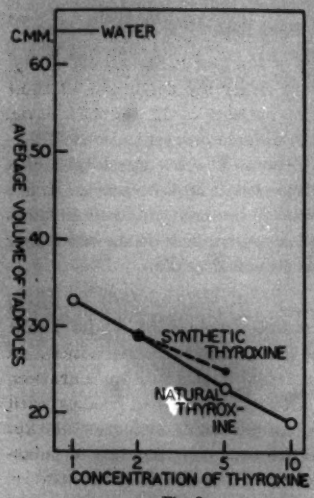


Fig. 3.

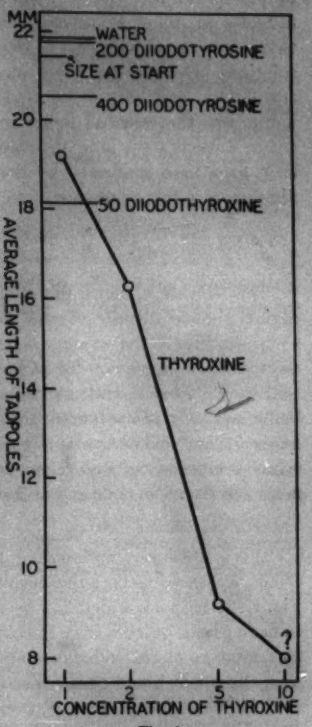
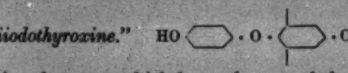


Fig. 4.

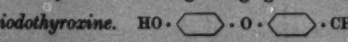
Fig. 3. Measurements taken on May 5th of the volume of tadpoles which had hatched on April 12th, and been placed in solutions of thyroxine for 48 hours on April 27–29th. The volume of the control batch is given at the top of the diagram. The concentration is given in parts per ten million. It was plotted on logarithmic paper.

Fig. 4. Measurements of the average length of batches of 12 tadpoles 46 days old, 7 days after an immersion in solutions of synthetic thyroxine, diiodotyrosine and "diiodothyroxine." The reading for the highest concentration of thyroxine should be neglected because many of the tadpoles were dead. The concentration is given in parts per ten million.

tadpoles. He also produced the characteristic effect by immersing the tadpoles in dilute solutions (4×10^{-7} for normal and $1{\cdot}1 \times 10^{-6}$ for thyroidectomised tadpoles) of the element iodine. He found that these solutions produced the effect more rapidly than thyroid preparations themselves, and he was led to the conclusion that the absorption of iodine was the essential factor in the development of metamorphosis. Other workers have failed to obtain these effects (see Abelin (11)), but they have been confirmed by Huxley (10), and it is probable that the differences in the results are to be attributed to differences in technique.

"Diiodothyroxine." 

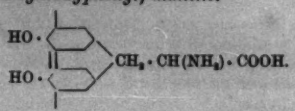
This substance, which has only two of the four iodine atoms of thyroxine, showed definite thyroid activity under the conditions of these tests. In Fig. 4 the average length of batches of 12 tadpoles seven days after an immersion for 24 hours in different concentrations of thyroxine is plotted against the concentration. The average length of a batch similarly treated with "diiodothyroxine" is also shown. It will be seen that "diiodothyroxine" appeared to be active in about 40 times the concentration of thyroxine. In other experiments it showed rather more activity. The counting of legs gave similar results.

Desiodothyroxine. 

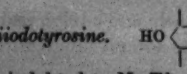
This substance, representing thyroxine deprived of all its iodine atoms, appeared to be quite devoid of the specific thyroxine action. In Fig. 1 the tadpoles which had been immersed in this substance are seen to have grown quicker than the controls. Those which had been exposed to a mixture of this substance and potassium iodide also grew quicker than those which had been exposed to the same concentration of potassium iodide alone. It appears, therefore, that tadpoles do not synthesise thyroxine under these circumstances.

In another experiment this substance had no effect on the rate of appearance of the legs.

ββdi (3, 5 *diiodo-4-hydroxyphenyl*) *alanine.*



This isomer of thyroxine showed no activity, as can be seen in Figs. 1 and 2.

3, 5 *Diiodotyrosine.* 

(I am indebted to Mr Thorpe for a sample of this substance.)

Under the conditions of these tests this substance produced little if any effect in high concentrations. In Fig. 4 it is seen to have less than one-eighth of the activity of "diiodothyroxine." In Fig. 1 it has less activity than the same concentration of potassium iodide and less than one-thousandth of that of thyroxine.

This result does not conflict with the results of previous workers. It is generally agreed that diiodotyrosine affects tadpoles like thyroxine when it is given over long periods and in high concentrations (see (5) p. 159 and (12)), but it has not been found to produce the effects after a single short immersion (compare the paper by Zawadowsky and his co-workers (13)).

Amines.

Experiments were done with the amines (amino-acid *minus* CO_2) corresponding to thyroxine and "diiodothyroxine" which seemed to indicate that they had a definitely specific effect similar to that of the same concentration of the corresponding amino acids; but it was necessary to add a small amount of alcohol and acid in order to get these preparations into solution, and it was found that the concentrations of these additions required were by themselves not without effect on the growth of the tadpoles, so that the results do not afford a very accurate comparison. In this connection it is interesting to note that Abelin (14) found that diiodotyramine had an effect similar to that of diiodotyrosine.

Discussion.

It is thought that these experiments indicate that the decrease in length which occurs in tadpoles some days after an immersion in a solution of thyroid preparations is a specific quantitative test for thyroxine. The only other substance tested which definitely showed activity comparable with that of thyroxine, though much smaller, was "diiodothyroxine," and it is possible that this is iodised directly by the tadpoles. Swingle's results seem to point to the conclusion that this specificity depends on the fact that iodine can be more rapidly absorbed and stored in a stable form when it is present as thyroxine than when it is present in other forms. Much of the iodine absorbed from solutions of potassium iodide was presumably excreted rapidly when the tadpoles were placed in tap-water again, so that the effect was only temporary.

I wish to thank Dr H. H. Dale for his help and advice, and also my assistant F. F. McDonald, who has made several thousand measurements of tadpoles.

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THE IMMEDIATE PRODUCTS OF POST-MORTEM GLYCOGENOLYSIS IN MAMMALIAN MUSCLE AND LIVER.

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In the present investigation evidence is furnished to show that carbohydrate substances which are intermediary between glycogen and lactic acid are formed during post-mortem glycogenolysis in the muscles. It is also shown that this process yields different end products in the muscles as compared with the liver.

That intermediary substances are formed in muscle has already been shown by several investigators, among whom may be mentioned Laqueur(1) and Lohmann(2). Laqueur found that the total yield of lactic acid could exceed that possible from pre-existing glycogen and lactacidogen, and Lohmann has succeeded in identifying as a triose the dialysable, yeast-unfermentable reducing substance which is formed by the action of muscle extract on glycogen. This triose is not, however, further split into lactic acid.

Because of the possibility that formation of such intermediary substances might, in part at least, account for the disappearance of sugar from the organism during the action of insulin, warm blooded animals have been used in the investigation in preference to cold blooded, on which insulin acts very slowly. The first step consisted in finding the conditions under which minimal values for lactic acid could be obtained in mammalian muscle. In the earlier experiments rabbits were used, the animals being killed by stunning. The legs were immediately skinned and pieces of muscle removed as quickly as possible and frozen in liquid air. The time from stunning of the animal to complete freezing of the muscle was less than two minutes. After being ground to a fine powder in an ice-cold iron mortar, samples of the frozen muscle powder were weighed out in previously cooled and tared tubes. An equal volume of 0·9 p.c. sodium chloride solution was then added and the tubes let stand in a water bath at 25° C. Sample tubes were taken every few minutes and the contents precipitated with hydrochloric acid-mercuric chloride

solution. Lactic acid was then determined by the von Fürth-Charnass method, after precipitation with $CuSO_4$ and milk of lime as recommended by Clausen, and in another portion of the solution phosphorus was measured by the Doisy-Bell method. Free sugar was determined in a second portion of the muscle powder by macerating it in 70 p.c. alcohol at -2 to $-3°$ C., and centrifuging, the residue being similarly treated several times until no more reducing substance could be detected in the extract. The supernatant fluids were combined, evaporated to dryness at low temperature, the residue dissolved in water and the solution mixed with a solution of lead acetate and alumina cream until no further precipitation occurred. Excess of lead was then removed by adding a few crystals of potassium oxalate and, after centrifuging, the reducing power of an aliquot portion of the clear supernatant fluid was determined by the Shaffer Hartman method. The control samples were mixed with the reagents immediately after weighing, while the muscle was still in a frozen state.

The controls of the first two rabbits showed "initial" muscle lactic acid contents of 0·388 and 0·353 p.c. respectively, indicating, in view of the low resting lactic acid level of amphibian muscle observed by Fletcher and Hopkins, that considerable post-mortem change had already occurred. In order to reduce this change to a minimum and to find the normal resting lactic acid level for mammalian muscle, experiments were performed in which great pains were taken to excise the muscle as quickly and with as little cutting as possible, and as our technique became better and better, lower and lower lactic acid values were obtained, viz. — 0·239, 0·198, 0·173 p.c.

Evidently the process by which the acid is produced is one that occurs with lightning rapidity immediately after death, and as we had reached the shortest time possible for removal of the muscle it became necessary to look for a method in which the muscle could be removed without previous disturbance of its blood supply. Knowing that the percentage of glycogen remains constant in the muscles of decapitated cats after they have been dissected free from their neighbours without disturbance of their blood supply, it was decided to use such preparations. The gracilis and semitendinosus muscles were chosen, their removal after dissection being very quickly effected by snipping across the insertions. They were then plunged in liquid air, ground to a powder and analysed as described above. By this method we have been able in one observation to obtain resting values for lactic acid as low as 0·024 p.c., so that it seems probable that the normal resting lactic acid in mam-

malian, as in frog muscle, is only a bare trace. In most of our observations the initial lactic acid content varied between 0·050 and 0·090 p.c. Glycogen under these conditions varied between 0·6 and 0·8 p.c., so that even in our best rabbit experiments, in which the muscle was removed and frozen in liquid air within a minute, the process of destruction must already have progressed to a considerable degree. Similar low lactic acid and high glycogen values for resting mammalian muscle have been published by Beatty and Milroy(3). These authors also emphasise the importance of rapidity in technique in order to obtain true results.

The first table shows the relationship between the rates of breakdown of glycogen and the appearance of lactic acid, free sugar and phosphoric acid in excised *rabbit* muscle kept after thawing at a temperature of 25° C.

TABLE I. Post-mortem changes in rabbit muscle.

	Time	Glycogen p.c.	Lactic acid p.c.	Free sugar p.c.	Phosphorus p.c.
I.	Immediate	0·240	0·388	0·134	0·107
	10 min.	—	0·669	0·146	0·114
	15 „	0·024	—	—	—
	20 „	—	0·671	0·145	0·121
	40 „	0·006	0·665	0·150	0·127
	60 „	0·003	0·710	0·163	0·128
II.	Immediate	0·308	0·353	—	0·110
	5 „	0·091	0·488	—	0·104
	10 „	0·053	—	—	—
	20 „	0·012	0·700	—	0·128
	30 „	zero	0·710	—	0·133
	60 „	zero	0·718	—	0·139

The high initial lactic acid, as well as the low initial glycogen, as compared with the corresponding values found when decapitate preparations were used, shows that the breakdown process had already proceeded well on its course by the time the tissue was fixed in the chemical reagents. The subsequent speed of glycogen disappearance was also very great, as is shown by a drop of 200 mgm. in five minutes (Rabbit no. 2).

The change in the percentage of free sugar was very slight and came about slowly and gradually, evidently bearing no relationship to the disappearing glycogen. The increase in free phosphorus was also a slow and steady one and might be considered as due to break-down of hexose phosphate, although in view of the work of Eggleton and Eggleton and of Fiske and Subbarow, this part of the work requires repetition.

In both experiments, as in several others of a similar nature, more lactic acid accumulated than could be accounted for by the glycogen which disappeared, thus confirming Laqueur. This excess of lactic

acid comes in all probability from intermediary substances arising as a first stage in the breakdown of glycogen, a process which must have been proceeding extensively before the "immediate" samples were taken, as evidenced by the low initial values for glycogen and the high ones for lactic acid.

Observations on cat muscle. In these the immediate values for glycogen and lactic acid show, for reasons already given, that post-mortem changes had not proceeded to any great extent by the time the muscle was frozen and ground up. In most of the observations the ground muscle, after mixing with Locke's solution, was kept *in vacuo* at 25° C. for 20 min. In others, the muscle was exposed to air and typical results of both groups of observations are shown in Table II.

TABLE II. Post-mortem changes in cat muscle.

		Initial p.c.	Final (20 mins.) p.c.	Phosphorus increase p.c.	Lactic acid increase p.c.	Glycogen decrease p.c.	Balance mgms.
I. *In vacuo.*							
1.	Lactic acid	0·055	0·420	—	0·365	—	—
	Glycogen	0·533	0·000	—	—	0·533	168
	Phosphorus	0·117	0·132	+0·015	—	—	—
2.	Lactic acid	0·070	0·436	—	0·366	—	—
	Glycogen	0·503	0·048	—	—	0·455	89
	Phosphorus	0·110	0·135	+0·025	—	—	—
3.	Lactic acid	0·065	0·402	—	0·337	—	—
	Glycogen	0·507	0·056	—	—	0·451	117
	Phosphorus	0·111	0·119	+0·008	—	—	—
4.	Lactic acid	0·059	0·378	—	0·319	—	—
	Glycogen	0·520	0·158	—	—	0·362	43
	Phosphorus	0·103	0·114	+0·011	—	—	—
II. In air.							
5.	Lactic acid	0·030	0·160	—	0·130	—	297
	Glycogen	0·804	0·377	—	—	0·427	—
	Phosphorus	0·113	0·104	−0·009	—	—	—
6.	Lactic acid	0·054	0·243	—	0·189	—	190
	Glycogen	0·633	0·254	—	—	0·379	—
	Phosphorus	0·116	0·146	+0·030	—	—	—
7.	Lactic acid	0·054	0·118	—	0·064	—	—
	Glycogen	0·793	0·328	—	—	0·465	461
	Phosphorus	0·115	0·115	0·000	—	—	—
8.	Lactic acid	0·040	0·220	—	0·180	—	350
	Glycogen	0·795	0·265	—	—	0·530	—
	Phosphorus	0·103	0·115	+0·012	—	—	—

Referring first to the observations *in vacuo* it can be seen that, with one exception, the glycogen had very largely disappeared in the 20 min. during which the muscle stood after thawing, but that lactic acid had not increased to a corresponding degree, the extent of the difference

being shown by the figures of the last column. This confirms the results obtained on rabbit muscle in showing that some intermediary substance must be formed. The small, though somewhat irregular increase in phosphorus does not support the view that this intermediate substance may be of the nature of lactacidogen, for if such were the case the free phosphorus would presumably be less, instead of greater, at the end of the observation. Of course it is possible that all available free phosphorus was already used up at the time the muscle was frozen and that, as the hexose phosphate formed from it was subsequently broken down, the liberated inorganic phosphorus was immediately used to form new hexose phosphate. In such a case the balance of free phosphorus at the time the post-mortem process was terminated might be practically the same as in the beginning.

In the observations in air the initial glycogen figures were decidedly higher than those *in vacuo*, but this we believe is merely a coincidence. The amount of glycogen which disappeared in 20 min. was practically the same in air as *in vacuo*, the average of the four experiments of both groups being 0·450 p.c. The increase in lactic acid, on the other hand, was decidedly less marked in the "air" experiments (average 0·347 p.c. "*in vacuo*," as compared with 0·141 p.c. "in air"). When this difference was first noted we thought that it might be of significance as indicating that a certain amount of lactic acid was removed by some oxidative process, but in the light of more recent experiments we are very doubtful of this explanation. In the experiments referred to we have compared the amounts of lactic acid formed in two samples of the same ground muscle placed in tubes so arranged that either air or nitrogen could be bubbled through their contents during the period of incubation. As the following figures will show, Table III, equal quantities of lactic acid were

TABLE III. Post-mortem formation of lactic acid in muscle kept either in oxygen or nitrogen.

	Initial lactic acid p.c.	Final lactic acid: 20 mins. oxygen p.c.	Final lactic acid: 20 mins. nitrogen p.c.	Lactic acid increase: In oxygen p.c.	Lactic acid increase: In nitrogen p.c.
1.	0·080*	0·500	0·475	0·420	0·405
	0·073	0·495	0·490		
2.	0·086	0·315	0·390	0·234	0·214
		0·325			
3.	0·058	0·400	0·395	0·324	0·327
	0·050	0·380	0·380		

* The duplicate analyses are given here to indicate the extent of the experimental error of the methods used.

formed in both tubes. We are at present at a loss to explain the greater formation of lactic acid in the vacuum tubes. During evacuation of these tubes apparently large bubbles of gas were evolved in the mixture. These were collected, but were not found to contain more than traces of CO_2. It is possible that the nitrogen used in the experiments of Table III may not have been strictly free of oxygen, since only the usual chemical procedure was employed to purify it.

The evidence which we have so far given for the presence of an intermediate substance between glycogen and lactic acid has been obtained, in most of the experiments, by comparisons made after glycogenolysis had proceeded almost to completion, and it is open to the criticism that the apparently greater disappearance of glycogen as compared with the appearance of lactic acid was really due to inaccuracies in the measurement of small traces of glycogen and of lactic acid. In the method used for the determination of lactic acid (Charnass-von Fürth) there is known to be an error which may amount to 10 p.c., so that if, in experiment 4 of Table II for example, in which the glycogen disappearance comes closest to the lactic acid formation, we add 10 p.c. to each of the lactic acid figures, the balance (last column) comes out at only 0·011 instead of 0·043. We have not applied this correction throughout, because we cannot be certain that it is constant and moreover, even if we did apply it, the majority of the balances would obviously still show a marked deficit in lactic acid formation as compared with glycogen disappearance.

Since the intermediate substance accumulates much more rapidly than lactic acid is formed from it, it must be present in relatively larger amounts at an early stage of the breakdown process than at later stages, such as those chosen (20 min.) in the above experiments. To demonstrate that this is the case we did several experiments in which the muscle was allowed to stand after thawing for only 5 min., by which time only about half of the original glycogen will have disappeared and possible errors due to estimation of traces of glycogen are eliminated. Typical results are shown in Table IV. In two out of the three experiments recorded more than twice as much glycogen disappeared as compared with the lactic acid which was formed, thus indicating that considerable quantities of the intermediate substance must be present during the earlier stages of glycogenolysis.

We have made no attempts to identify the nature of the intermediary substance. In using dissected cat muscles, as in the foregoing experiments, enough material was not available so that determinations of glycogen and lactic acid could be made at a sufficient number of intervals

TABLE IV. Post-mortem changes in cat muscle after standing five minutes at 25° C.

		Initial p.c.	Final (5 mins.) p.c.	Lactic acid increase p.c.	Glycogen decrease p.c.	Balance p.c.
1.	Glycogen	0·950*	0·535			
		1·000	0·535		0·440	
						0·205
	Lactic acid	0·099				
		0·090	0·330	0·235		
2.	Glycogen	0·860	0·465			
		0·850	0·450		0·398	
				0·166		0·232
	Lactic acid	0·050				
		0·068	0·225			
3.	Glycogen	0·680	0·325			
		0·695	0·290		0·380	
						0·209
	Lactic acid	0·082	0·252			
		0·089	0·262	0·171		

* Duplicates.

to permit of accurate time curves of the two processes being compared. We have already published a typical curve for the process in rabbit muscle[1], but since glycogenolysis must have been well on its way before the first estimations were made, little can be learned from the curve with regard to the possible accumulation of the intermediary substance. Neither have we as yet any data to show how closely lactic acid formation may come to glycogen disappearance in mammalian muscle when the post-mortem process is allowed to proceed for longer periods of time than 20 min.

Glycogenolysis in the liver. It is well known that sugar accumulates as glycogen disappears when liver is allowed to stand after death, but the methods for estimation of the sugar have been too inaccurate to make it possible to say whether the two processes run proportionately. In perfused liver it has been found by Seegen(4), Embden(5) and others that more sugar appears in the perfusate than can be accounted for by the glycogen which meanwhile disappears, and Burn and Marks(6) have recently given evidence which shows that the source of this extra sugar may be fat. There can be no doubt that the physiological significance of glycogen is fundamentally a different one in muscle and liver and it seemed of interest to determine, by methods similar to those used for muscle, whether accumulation of sugar and lactic acid during post-mortem glycogenolysis in the liver proceeded differently. This has been carried out for the liver of both the rabbit and the cat, and typical results are shown in Table V. The amounts of glycogen which disap-

[1] *Proc. Soc. exp. Biol. and Med.* 23. p. 659, 1926. The values for lactic acid as given in the curve in the preliminary communication should have been multiplied by 10.

TABLE V. The behaviour of sugar, lactic acid and phosphoric acid during post-mortem glycogenolysis in the liver.

Animal		Percentages in minutes after thawing liver powder: Immediate	20	30	60	90	115–120	180
Rabbit 1	Glycogen	4·60	—	3·65	—	2·53	—	1·59
	Lactic acid	0·02	—	0·02	—	0·02	—	0·01
	Free sugar	0·12	—	1·04	—	1·72	—	—
Rabbit 2	Glycogen	1·52	—	0·96	—	—	0·71	0·47
	Free sugar	0·11	—	0·44	—	—	0·37	0·93
Rabbit 3	Lactic acid*	0·02	—	0·02	—	0·02	0·02	0·02
Rabbit 4	Lactic acid*	0·03	—	0·01	—	—	0·02	0·01
Rabbit 5	Lactic acid	0·07	0·05	0·05	0·05	—	—	—
	Free sugar	0·26	0·67	0·84	1·13	—	—	—
Cat	Lactic acid	0·04	—	—	0·05	—	0·04	0·03
	Phosphorus	0·04	—	—	0·05	—	0·06	0·06

* The liver stood *in vacuo*.

peared per 100 grm. of liver in the first 30 min. was 0·95 grm. and 0·56 grm. respectively in the two experiments in which it was measured, the rate of disappearance becoming progressively less during each of the subsequent periods. Studies of the exact rate of this process at later stages have already been published by one of us (J. J. R. M.)(7). The absolute amount of glycogen which disappeared in unit time was greater than in muscle, but in striking contrast to muscle, free sugar increased rapidly whereas lactic acid remained practically unchanged. In No. 1, for example, 2·07 grm. of glycogen per 100 grm. of liver disappeared in 90 min. but only 1·60 grm. of sugar accumulated; in No. 2, 1·05 grm. glycogen disappeared and 0·82 grm. sugar appeared. It is possible that much closer agreement between glycogen disappearance and sugar accumulation would be obtained if a more certain method could be devised for determination of the free sugar in tissue. The method employed (see p. 256) is probably suitable enough for comparative studies of the behaviour of the sugar itself, but it is probably not so for the measurement of absolute amounts of sugar.

The results as they stand are quite sufficient to show, in so far as can be judged from the immediate post-mortem process, that the fate of glycogen in the liver is an entirely different one from that in muscle. Once it has become deposited in muscle, glycogen may be considered to have entered into an irreversible reaction, in that its only breakdown pathway is by way of lactic acid; and it is impossible for it again to form glucose unless in cases in which the oxygen supply is inadequate for its oxidative synthesis into glycogen, when some will enter the blood and

be re-formed into glycogen (glucose) in the liver. In support of this view may be cited the fact, first observed by Mann and Magath and confirmed by Soskin, that the blood sugar does not become increased when epinephrin is injected into animals deprived of the liver, or when such animals are asphyxiated, even although abundance of glycogen is still present in the muscles.

The effect of insulin on the free sugar of the muscles and liver.

Although of a somewhat different nature we wish to include in this paper certain observations which we have made pertaining to the behaviour of free sugar in the liver and muscles following the injection of insulin. Observations of a similar nature have already been published both from this laboratory and by Cori and Cori(8), but certain objections can be raised to the results.

The standard white rat was the animal chosen in the present observations. After fasting for 24 hours it was decapitated and the liver and muscles immediately excised and frozen in liquid air. The hard frozen tissue was then ground to a dust-fine powder, of which weighed quantities were thoroughly shaken with ice cold alcohol (70 p.c.) and then rapidly centrifuged. These extractions were repeated until no reducing substance could be detected in the evaporated alcoholic extract. The reducing power of a solution of the residue of the combined alcoholic extracts was found to be very similar for different rats (about 0·540 p.c.) and was very close to the value reported as free sugar by Cori and Cori. But of course this cannot all be free sugar, for if it were so then the liver cell would contain more than five times as much free sugar as the blood, indeed much more than this if we allow for the blood present in the liver. After trial of various methods for the removal of the interfering substances (tungstic acid, phosphotungstic acid, etc.) we finally decided to use neutral lead acetate and alumina cream, the actual proportions used being as follows: the final residue from 2 grm. of liver powder was dissolved in 6 c.c. of water in a centrifuge tube and after centrifuging 2 c.c. were pipetted off and mixed in another centrifuge tube with 0·2 c.c. of a saturated solution of neutral lead acetate and 0·4 c.c. alumina cream. After standing, a drop of the lead acetate solution was added to make certain that precipitation was complete. Excess of lead was then removed from the solution by shaking with a trace of potassium oxalate, the volume was made up to 5 c.c., centrifuged and 2 c.c. of the supernatant fluid used for sugar determination. It will be seen from

Table VI that a large amount of reducing substance is removed from the alcoholic extracts by the above treatment, and we cannot be certain that

TABLE VI. Free sugar in liver of normal starved rats.

	Direct determination		After lead acetate and alumina cream	
Date	1st extract	Total	1st extract	Total
Nov. 6	0·272	0·530	0·048	0·085
	0·273	0·549	0·046	0·077
Nov. 12	0·266	0·533	0·089	0·131
	0·275	0·579	0·074	0·084
Nov. 17	0·279	0·584	0·066	0·072
	0·248	0·581	0·060	0·065
Nov. 19	0·244	0·460	0·089	0·138
	0·247	0·475	0·066	0·076
Averages:	0·263	0·538	0·067	0·079

All animals starved for a period of 18–24 hours previous to experiment.

all of the reducing substance which remains is glucose. Although, as certain of the duplicates show, this method cannot be considered as quantitative our results are much more likely to represent the true glucose concentration of the liver than are the much higher ones of other workers, since they correspond much more closely to the values for blood sugar. Thus, the average percentage of glucose in the blood of a rat starved 24 hours may be taken as 0·100 and that of our liver observations is 0·067. Actually the percentage in the liver cells must be considerably less than this, since much blood is contained in the viscus when it is frozen. It is impossible to calculate what the actual percentage in the liver cell may be, because we do not know the amount of blood. The reagents used do not precipitate any glucose, as can readily be shown by adding a measured quantity to the solution of the alcoholic extract and then precipitating with lead acetate and alumina cream when all the added glucose is recovered.

When insulin was injected into the rats in amounts sufficient to bring the blood sugar to about the convulsive level, Table VII, the free liver sugar was markedly reduced although very little change could be detected in the reducing power of the extract before treating it with lead acetate. That insulin reduces the free sugar of the liver has already been observed by Cori and Cori, the method of extraction being one, however, in which there was probably some hydrolysis of glycogen (extraction with ice-cold 2 p.c. HCl and subsequent treatment of the extract with mercuric chloride). Insulin therefore reduces both the free sugar and the glycogen of the liver when it is given to fasted rats

TABLE VII. Free sugar in liver of starved insulin-treated rats.

Date	Direct determination: 1st extract	Direct determination: Total	After lead acetate and alumina cream: 1st extract	After lead acetate and alumina cream: Total	Blood sugar p.c.	Remarks
Nov. 24	0·255	0·540	0·039	0·039	—	Starved 24 hrs.
	0·255	0·585	0·039	0·038	—	½ unit insulin.
Nov. 26	0·264	0·487	0·010	0·010	0·040	Starved 24 hrs.
	0·263	0·508	0·011	0·011	—	1 unit insulin.
Dec. 1	0·294	0·517	0·010	0·010	0·030	Starved 24 hrs.
	0·276	0·512	0·010	0·010	—	1 unit insulin.
	—	—	—	0·049	0·070	Starved 24 hrs. 1 unit insulin.
	—	—	0·055	0·055	—	Animal recovering.
Averages:	—	0·524	0·031	0·031	—	—

Note. All animals starved for a period of 18–24 hrs. previous to experiment.

(cf. Barbour, Chaikoff, Macleod and Orr) (9), and it is possible that the mechanism by which it does so is inhibition of gluconeogenesis, although it is at present impossible to obtain direct evidence that this occurs in normal animals. It is perhaps significant in this connection that the greatest reduction in free sugar occurred in the two rats of our series in which the blood sugar became most markedly depressed.

The results for muscle, using exactly the same methods as for liver, are also shown in Table VIII, and it can be seen, in confirmation again of

TABLE VIII. Free sugar in rat muscle.

Date	Total Direct	Total after lead	Blood sugar p.c.	Remarks
Jan. 7	0·206*	0·045	—	Rat starved 24 hrs.
	0·199	0·038	—	
Jan. 12	0·245	0·051	—	Rat starved 24 hrs.
	0·260	0·052	—	
Jan. 14	0·250	0·051	—	Rat starved 24 hrs.
	0·238	0·030	—	
Jan. 19	0·190	0·055	0·065	Rat starved 24 hrs.
	0·230	0·055	—	1 unit insulin, but no hypoglycaemic symptoms.
Jan. 21	0·415	0·039	0·055	2 unit insulin, definite hypoglycaemic symptoms.
	0·420	0·048	—	
Jan. 26	—	0·042	0·058	2 unit insulin, definite hypoglycaemic symptoms.
	—	0·039	—	

* Duplicates.

Cori and Cori, that insulin does not cause the free sugar to become lowered. These workers used for the muscles a somewhat different method for determination of the free sugar from that which they used

for the liver (boiling water extraction and removal of interfering substances with colloidal iron and Lloyd's alkaloidal reagent) and their average results are higher than ours (0·079 p.c. as compared with 0·045 p.c.). The failure of insulin to lower the concentration of free sugar of muscle is very difficult to explain, but it is significant that the result harmonises with the absence of any change in that of glycogen, under similar conditions. It has been customary to assume that glucose disappears from the blood following insulin because of its more rapid diffusion into tissues (muscles) in which its concentration has become lowered (glucatonia). The results of both Cori and Cori and ourselves do not lend support to this view.

Conclusions.

1. In mammalian muscle which is frozen and ground up in liquid air immediately after its removal from a living animal (decapitated cat) and then allowed to stand at room temperature, glycogen almost entirely disappears within 20–30 min.

2. Lactic acid does not appear at the same rate as glycogen disappears, indicating the presence of intermediary products.

3. There is only a slight increase in free sugar and inorganic phosphorus while glycogenolysis is proceeding.

4. Entirely different results are obtained when the liver is studied by the same methods, for in this case free sugar accumulates almost in proportion as glycogen disappears, and lactic acid does not increase.

5. Insulin causes the free sugar of the liver to be decreased but has no effect on that of the muscles.

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CHANGES IN OXYGEN CONSUMPTION DURING METAMORPHOSIS INDUCED BY THYROID ADMINISTRATION IN THE AXOLOTL.

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When we consider the degree of organolysis and of morphological changes in general which accompany amphibian metamorphosis, it would seem evident that the rate of metabolism must also alter considerably during the process. Nevertheless, the small amount of evidence on this point is not wholly concordant. Asher(1) has put forward the hypothesis that the thyroid in amphibian larvæ provokes metamorphosis by increasing the need for oxygen; the animal is thus led to migrate from water to air. Gayda(3) has found that the production of heat is markedly increased in toad tadpoles fed with thyroid and that no such effect can be produced in animals fed with thymus or meat. At 18°, the increase under the action of thyroid reaches its maximum on the sixth day, at 22·5° on the fourth day. The maximal increase is about 25 p.c. at 18°, 45 p.c. at 22·5°. Abelin and Scheinfinkel(3), on the other hand, state in a more recent paper that CO_2-output increases immediately after administration of thyroid to frog tadpoles and *Amblystoma* larvæ, but that during metamorphosis itself the CO_2-production diminishes, finally reaching only 50–70 p.c. of the original normal larval value. They did not, however, take into account the important decrease of body-weight which occurs during metamorphosis, and their values are therefore calculated per animal and not per unit of body-weight. From the experimental data published by Drastich(4) it may be seen that the CO_2-production as well as the oxygen intake diminishes progressively during the larval development of *Salamandra maculosa*, but that there is a sudden increase at the time of natural metamorphosis. Helff(5) too, in a recent paper, states that the oxygen-requirements of Anuran tadpoles are increased both in normal and induced metamorphosis; see also Huxley(10). The considerable loss of weight, which takes place during metamorphosis, points to the same conclusion. It is clear that further work is desirable to elucidate the

mechanism by which the thyroid affects the general metabolism of amphibian larvæ.

A. *Method.*

In our work we used Axolotls, *i.e.* larval *Amblystoma tigrinum*. This species was chosen because of the possibility of performing individual experiments and of dosing exactly the amount of thyroid administered per unit of body-weight. We have employed specimens both of the black and white varieties, of small medium size, weighing 9·3–15·5 grm. at the moment of first thyroid injection. The animals were kept at a constant temperature of 23° C.

The rate of metabolism was determined as oxygen consumption per unit of time and of body-weight. Determinations were made by means of a modified small Haldane apparatus, with flat-bottomed bottles of various sizes, the largest sufficient to contain axolotls of 30 grm. The temperature of the water-bath surrounding the apparatus was kept constant at 23° C. by means of a mercury-toluol gas-regulator. A special form of shaker for slow gentle shaking by means of excentrics was devised.

Irregularities due to variations in muscular activity had to be avoided, and it was found that this could be done without materially affecting respiratory movements or heart-beat by keeping the animals under light anæsthesia in a solution of amytal, 1 part in 3000 of tap-water. This condition will be referred to as "standard anæsthesia." In this way the oxygen consumption was reduced to 21–30 c.c. per kg. per hour as compared with 35–97 without the anæsthesia, but otherwise under the same conditions. The amytal was kindly supplied to us by The Eli Lilley Co. of Indianapolis, U.S.A. Amytal we found superior to urethane, though this also gave fairly satisfactory results. For each animal, determinations were made both in normal conditions and under "standard anæsthesia."

Metabolism was found to be increased after food (see Krogh(6)), the effect beginning within an hour but being still perceptible after 24 hours. The following figures show this:

	c.c. O_2 per hour and kg.	
	No. 7 anæsthetised	No. 2 not anæsthetised
Before feeding ...	29	52
1 hour after feeding	35	76
3 hours after feeding	42	76
5 " " "	43	84
24 " " "	31	59

We have therefore not used any determinations made less than 24 hours after the animal's last meal. On the other hand, prolonged starvation caused a progressive decrease in metabolism, but as to the rate of this change, considerable individual variation was noticed. In some individuals a marked decrease in metabolism (both standard and normal) occurred a few days after the beginning of starvation; and in these the body-weight decreased very slowly. In another group the metabolism diminished very slowly and fell to its minimal level only after some weeks; in these cases, however, the loss of body-weight was very rapid. It is obvious that the lowering of metabolism in starvation has in a sense an adaptive function, in permitting a less rapid loss of substance than would otherwise be the case. But it is further clear from our results that the adaptive value of the mechanism is highly variable, being much greater when there is a rapid initial fall of metabolic rate. At present one can only speculate as to the causes of these individual differences.

We found that the curve representing the decrease of body-weight in starved axolotls cannot be expressed by the usual formula, according to which the rate of decrease is proportional to the remaining amount of living substance (see Morgulis(8)).

For these reasons some of the animals were kept during the experiments without any food, a treatment which the axolotl withstands without injury for 8 to 12 weeks, and others received only occasionally (one to three times a week) small amounts of veal, just sufficient to keep their weight constant.

The thyroid used in our experiments was Armour's "Tabloid" preparation. To prepare this for injection six tablets (each equivalent to 0·2 grm. of dry gland) were dissolved in 40 c.c. of 0·7 p.c. NaCl, made slightly alkaline by adding one to two drops of *N*/10 KOH, and heated rapidly to boiling. After cooling at room temperature and adding water to counterbalance evaporation, the solid particles were thrown down by light centrifugation. The supernatant clear solution was injected undiluted, or, if necessary, further diluted with saline. Injections were made intraperitoneally and were usually repeated at intervals.

For each animal determinations were made both in normal conditions and under standard anæsthesia and several determinations were carried out before any injections were given.

The animals were regularly weighed (to 0·1 grm.) after being dried with a soft cloth.

B. *Results.*

In Figs. 1–6 we give individual curves of body-weight and normal and standard metabolic rates. Descriptions of these curves are given

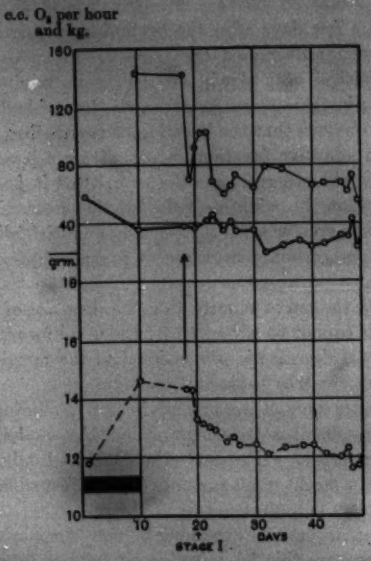

Fig. 1. In all figures, thin continuous line, normal (unanæsthetised) metabolism; heavy continuous line, standard (anæsthetised) metabolism; broken line, body-weight; black band below, periods when fed. Arrows denote injections. Roman figures in circles denote stages in metamorphosis as defined in text.

Axolotl, no. 1. Injected after 6 days' starvation with < 24 mg. dry gland (= < 8·3 mg. fresh gland per grm. body-weight). Metamorphosis did not proceed beyond stage I. Exophthalmos persisted till death eight months after injection. No increase of metabolic rate due to thyroid.

in the legends appended. As mentioned above, most of the animals were kept in a state of complete or partial starvation during the experiment. The effect of this may be seen in the curves of body-weight as well as in those of metabolic rate. In some instances the injections began before the starvation-equilibrium was reached. In such cases, decrease of body-weight after injection can only be regarded as due to the thyroid

when the body-weight curve shows a marked change of slope. In the same way, increase of metabolism due to thyroid must be considered relative to the level of metabolism at the time. A marked rise of metabolism during metamorphosis may not bring the O_2-consumption

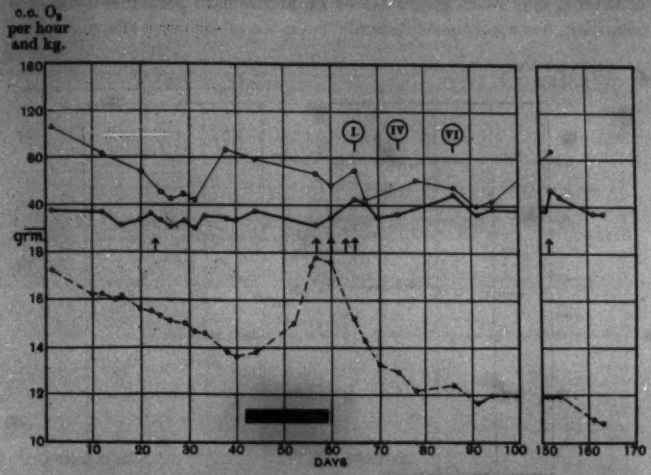

Fig. 2. Axolotl, no. 4. The first injection, after 3 weeks' starvation, was of 13 mg. dry gland (= approx. 4 mg. fresh gland per grm. body-weight). The next four, each of 2 mg. dry gland (= approx. 0·5 mg. fresh gland per grm. body-weight). First injection, though large, and producing an increase of weight loss, was without effect on metamorphosis or metabolism. The next four had a marked effect on body-weight and metamorphosis, with progressive increase of standard metabolism, sinking again after metamorphosis. When injected after complete metamorphosis, *immediate* effect on standard metabolism.

up to the original larval level obtaining before starvation began. In the records, metamorphic changes are classified as follows:

Stage: I. Exophthalmos; reduction in height of fin on back.
II. Resorption of the whole height of the dorsal fin:
(*a*) Beginning;
(*b*) Resorbed half-way along the back;
(*c*) All along the back.
III. Gill-filaments totally resorbed.
IV. Gill-axes reduced to small knobs.

V. Moult of larval skin.

VI. Closure of gill-slits and emergence to air.

Stage V appears to be the critical stage, after which metamorphosis may be considered complete. Animals treated with an insufficient amount of thyroid may reach stages III or IV and remain permanently in this condition, metamorphosis thus not being an all-or-none effect.

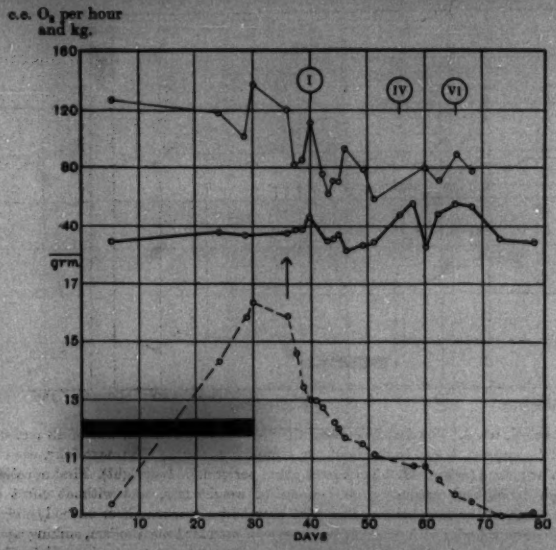

Fig. 3. Axolotl, no. 5. Injected, after 6 days' starvation, with 12·5 mg. dry thyroid (= approx. 4 mg. per grm. body-weight). Marked (nearly 50 p.c.) decrease of body-weight. Standard metabolism rises markedly towards the close of metamorphosis, beginning with stage II(b), and falls to normal after its completion. (After complete metamorphosis, 11 injections, each = approx. 5·5 mg. fresh gland per grm. body-weight, were made during four weeks: the standard metabolism remained high throughout.)

The doses of thyroid administered are given in terms of dry as well as of fresh gland. In some injections a few drops were lost during the manipulation; in these cases < is prefixed to the dose.

In addition to the results recorded graphically in the figures, Axolotl no. 3 (10·9 grm. in weight) was injected after 3 weeks' starvation with the equivalent of 8 mg. fresh thyroid per grm. body-weight

(13 January 1926). No significant changes in weight, metamorphosis, or metabolism were observed (standard metabolism 28–34 c.c. O_2 before, 26–33 c.c. O_2 after the injection). On 18 February accidental death occurred; no metamorphic changes. This animal was unusually resistant to thyroid.

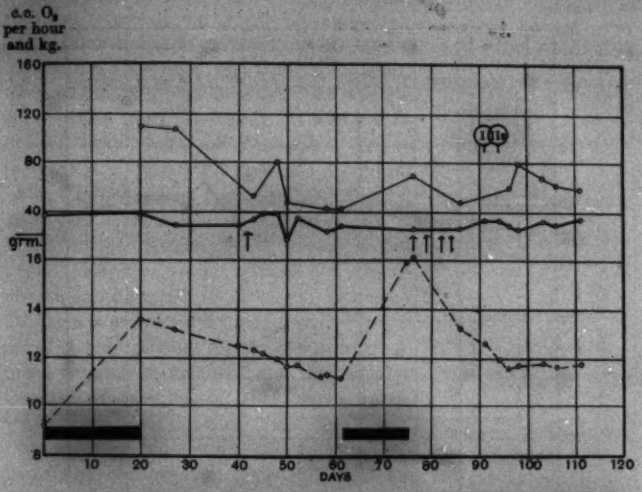

Fig. 4. Axoloti, no. 6. After 3 weeks' starvation, injected with approx. 16 mg. dry gland (= approx. 6 mg. fresh gland per grm. body-weight). Only slight increase in rate of weight-loss, and no increase in standard metabolism. Four later injections each equivalent to 0·25 mg. fresh gland per grm. Marked decrease in body-weight: incomplete metamorphosis. No rise in standard metabolism. Seven months later stages III and IV were reached; standard metabolism normal.

C. *Discussion.*

As may be seen from the graphs, the normal metabolic rate is by no means always proportional to that determined under narcosis. When we consider the various external influences which may influence normal metabolism, and remember that these influences may be partly or completely excluded when the animal is anæsthetised (see Krogh(6)), it is obvious that a standard metabolic rate under known degree of anæsthesia should be taken as the best indicator of metabolic changes of internal origin. As a matter of fact, the "normal" unanæsthetised

metabolism will permit the study of such internal changes only under exceptionally favourable and well-controlled experimental conditions. The discrepancies found in the literature concerning metabolic changes in amphibian metamorphosis very possibly owe their origin to the fact

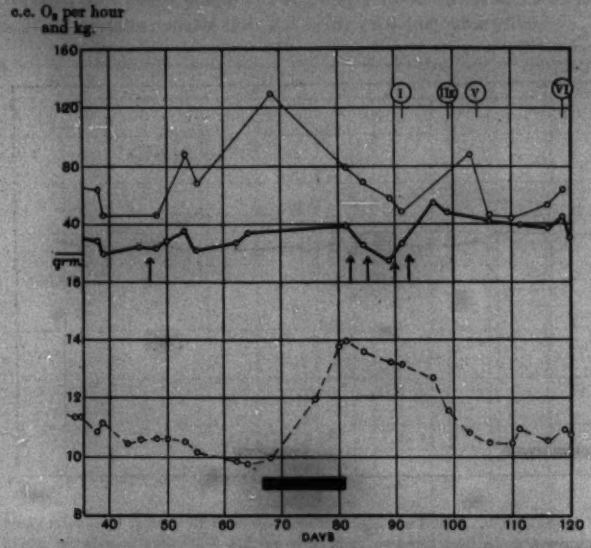

Fig. 5. Axolotl, no. 8. After 3 weeks' starvation, injection of equivalent of approx. 2 mg. fresh thyroid per grm. body-weight. Increase in normal metabolism, but hardly any in standard. No increase in rate of weight-loss. Later four injections, each equivalent to approx. 0·1 mg. fresh thyroid per grm. body-weight. These followed by rapid increase in weight-loss, and by metamorphosis. Decrease followed by marked increase in standard metabolism, falling to normal after complete metamorphosis. The feeding during the experiment rendered the standard metabolism, at the time of the second thyroid injection, above the normal.

that the respiratory exchange under ordinary conditions has usually been taken as a measure of internal metabolic processes, instead of that under a uniform degree of narcosis. Under the conditions of feeding, temperature, etc., mentioned above, the average value of standard metabolism was 30 c.c. of oxygen per kg. of body-weight per hour. Normal variations of this value did not exceed $\pm$ 10 c.c. O_2. Values

higher than 40 c.c. O_2 may be regarded as outside the normal range, although individual variation is so large as not to allow us to state this as an absolute rule.

When summarised from this point of view, our results show that thyroid-induced metamorphosis in the axolotl is accompanied by an increase of metabolic rate (as measured by oxygen consumption). This increase, however, never occurs immediately after injection, but takes place later, when the main effects of organolysis and reconstruction begin to appear. It seems that in the early stages of these processes no increase of metabolism is involved. In animal no. 1, Fig. 1, in which metamorphosis stopped at a very early stage, as well as in no. 6, Fig. 4, whose metamorphosis only proceeded about half-way, no significant increase of metabolic rate could be observed. The same also held good for no. 3 (not figured). On the other hand, already-metamorphosed specimens respond to thyroid by a very prompt increase of metabolism. The mean values for oxygen consumption, "standard" and "normal," for the various periods, and their percentage changes from period to period, are summarised in Table I.

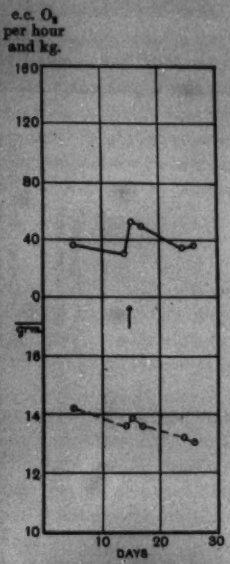

Fig. 6. *Amblystoma*, no. 9 (a previously metamorphosed specimen). After 8 days' starvation, injection of 7 mg. dry gland (= approx. 2·5 mg. fresh gland per grm. body-weight). Marked rise of standard metabolism on the succeeding day, dropping gradually to normal.

It seems therefore that the increased metabolism is not *per se* responsible for the occurrence of metamorphic processes taking place, because some at least of the latter can occur independently.

It is clear that during the long period after injection during which little or no change in metabolism is observed, important changes, necessary for metamorphosis, are going on. This is revealed first in the loss of weight, which may begin within the first day or so, and also in the exophthalmos and fin-reduction. It is remarkable that no increased oxygen-consumption accompanies the early weight-loss. It is further not clear whether the later rise in metabolism is to be regarded as the cause or the effect of the final morphological changes. It may perhaps

TABLE I.

Specimen number	Time to complete metamorphosis from 1st injection (days)	Marked rise of metabolism starts (days)	Reverts to normal (days)	"Standard metabolism" (c.c. O_2 per kg. per hour) A: Up to 1st injection av.	A: (max.)	B: Period before marked rise av.	B: (max.)	C: From marked rise onwards av.	C: (max.)	p.c. change A–B of av.	A–B (of max.)	p.c. change A–C of av.	A–C (of max.)
4	29	<8	>29 <34	28	(35)	29	(29)	39	(47)	3	(−17)	39	(34)
5	30	>14 <19	>30 <35	34*	(36)*	32	(45)	47	(57)	−6	(25)	38	(58)
8	37	>10 <15	38	29†	(36)†	22	(27)	45	(55)	−24	(−25)	55	(53)
9 (metamorphosed)	—	1	>2 <9	32·5	(36)	—		50	(52)	—		54	(44)
				Normal metabolism (not under narcosis)									
4	—	—	—	57	(86)	57	(69)	57	(87)	Negligible or irregular			
5	—	—	—	118*	(127)	79	(111)	80	(89)				
8	—	—	—	68†	(100)†	58	(68)	59	(88)				

* Omitting record of Dec. 22, because recently fed.

† Omitting records of Feb. 3 and Feb. 16, because fed.

be concluded that the changes during the refractory period bring about a changed physiological equilibrium which permits of response by heightened metabolism to thyroid substance and perhaps to other internal stimuli; and that, once gained, this faculty is retained (immediate metabolic response of metamorphosed *Amblystoma* to thyroid). It is also perhaps possible that thyroid substance is at first in some way fixed by the tissues showing morphogenetic changes, and that only when these have utilised what is necessary is the remainder available for increasing metabolism. At present it is not possible to decide whether these are special features of neotenic forms, or occur in amphibians in general. In any case, it is clear that the relation between the metabolic and the metamorphic effects of thyroid in *Amblystoma* is not so simple as might be expected.

Conclusions.

1. Significant changes in metabolic rates (oxygen-consumption) during metamorphosis of axolotl larvæ induced by thyroid can only be observed satisfactorily if the oxygen-consumption under definite degree of narcosis is taken as standard, since the normal metabolic rate is subject to too great a range of variation.

2. Metabolic rate, as determined by oxygen-consumption per unit of body-weight, is not affected immediately after injection of thyroid to larval axolotls, but is increased after a refractory period of about 8–14 days at 23° C. The increase in metabolism occurs at about the metamorphic crisis (shedding of larval skin) but a number of morphogenetic changes and considerable weight-loss occur before it takes place.

3. This increase of metabolism fails to occur when metamorphosis is incomplete, even when the gills are reduced to stumps, and the fin has disappeared from the trunk.

4. Metamorphosed *Amblystoma* do not show this refractory period. Under the same conditions they respond almost immediately to similar doses of thyroid by an increase in metabolic rate.

5. As a possible hypothesis, it is suggested that one of the important changes effected by thyroid injection during the refractory period is a change in physiological equilibrium which permits the animal to respond to internal stimuli such as thyroid substance by an increase in metabolism; and that this changed equilibrium is permanently retained.

6. Great variation is found in rate of weight-loss and of metabolism-decrease during total starvation. When rapid initial decrease of metabolism occurs, weight-loss is slight and gradual, and vice versa.

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THE ACTION OF LIGHT ON THE EYE. Part II. The processes involved in Retinal Excitation.

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In Part I of this series (1) we gave a general account of the discharge of impulses in the optic nerve of the conger eel when the eye is exposed to light. In the present paper we are concerned more particularly with the initial stages of the process, *i.e.* with the excitation of the retinal elements, and we shall have little to say about the subsequent nervous changes. The latter have shown themselves to be of great interest from the neurological point of view, but before we dealt with them at length it seemed worth while to try and gain a little more information about the action of light as a stimulus to the retina. We have attempted to do this by studying the effect of flashes of light of short duration. In Part I we were mainly concerned with relatively long exposures and it was possible that some of the phenomena we found might be considerably modified with flashes. In any case a study of the response to flashes ought to give us data bearing on retinal excitation and it ought, in particular, to show how far we can liken the mechanism of the vertebrate eye to that of the much simpler organs investigated with the same method by Hecht (2).

Method. The apparatus used differed little from that described in Part I with the exception of the shutter and the signalling arrangement. The shutter contained a slit of varying width moved by a short, heavy pendulum like that of the Lucas contact breaker. Two discs of 3-ply wood, 60 cm. in diameter, are carried on a horizontal steel shaft running in ball bearings. A block of lead weighing 6 kilos was bolted to one side of the shaft to form the pendulum. The lead block was lifted to one side to the same level as the shaft and then allowed to swing through 180° rotating the discs with it. Sectors were cut away from the periphery of the discs to form an adjustable slit of 3 mm. to 20 cm. width and the discs were set so that the slit admitted light to the eye chamber at the moment when the pendulum bob was vertically below the shaft, *i.e.* when the velocity of the discs was at a maximum. The illuminating system was a 500 c.p. pointolite lamp placed at a distance of 44 cm. from a short focus convex lens. The rays from the lens came to a point focus in the plane of the slit and then diverged again to form a patch of light on an opal glass disc placed 18 cm. in front of the eye. The disc was placed at such a distance from the lens and shutter that the illumination was as uniform as possible over a circle of 3 cm. diam. With this arrangement, even with a slit of only 3 mm. width, the disc was fully illuminated for

the greater part of the time of exposure. The intensity of the light was altered by placing a series of screens of different density in front of the lens. These were made of photographic plates exposed to light for different periods. Those generally used produced a reduction of the light to 40, 14 and 2·5 p.c. of its original intensity. The illumination of the opal glass screen on the side to which the eye was exposed was approximately 500 metre candles with no screen, 220 metre candles with the first, 78 with the second and 14 with the third. We express these illuminations as 100, 40, 14 and 2·5 units.

For signalling the exposure we used a modification of the optical method introduced by Gotch, in which the flash of light records itself directly on the moving photographic surface. A second slit was formed in the rotating discs equal in width to the first and placed so that it opened a narrow beam of light from a ¼ watt lamp at the moment when the first slit exposed the eye. The beam of light was thrown by a train of mirrors into the recording camera and made a dark line at the side of the electrometer record. Since both slits are rigidly connected and are of the same width, the appearance and disappearance of the signal mark on the film coincides with the period of illumination of the eye. With this signal the error is less than a thousandth of a second. The duration of each exposure is marked on the record but it was found that with a width of slit of 10 cm. or less the relation between the width and the length of exposure was practically linear. The following table shows the durations corresponding to the different settings in common use.

TABLE I.

Width of slit	Duration of flash (sec.)
8 inches (204 mm.)	·094
4	·0435
2	·0217
1	·0108
½	·0054
¼	·0027
(⅛; rarely used	·00135)

As in our earlier experiments the area of the illuminated disc was controlled by an iris diaphragm and its image was focussed on the retina by placing the necessary lenses in front of the eye. In all the experiments where the size of the image remained constant (pp. 281–8) the illuminated disc had an area of 1024 mm.^{2}, corresponding to a retinal image of ·95 mm. diam. The electrodes on the nerve were usually arranged for monophasic recording; this makes it difficult to count the individual responses in the discharge but has the advantage of giving a large initial excursion. Typical records of the response to flashes are given later in Fig. 7. The eyes were normally kept in the dark and intervals of a minute or more were allowed between successive exposures. These intervals seemed long enough to give consistent results.

The measurement with which we are particularly concerned is that of the "reaction time" of the optic nerve discharge or of the retinal current. By this we mean the period between the beginning of the exposure to light and the beginning of the nerve or retinal response. In our first paper we referred to this as the latent period, but as this term has been used in a different sense by Hecht it seemed better to avoid it. The present

experiments were carried out in July and August and the reaction times have been considerably shorter than those we found in the winter months. The temperature of the eye chamber was 2–3° higher and the eels were generally in a better condition.

Effect of intensity and duration of flash on nerve reaction time.

When the exposure is of long duration (1 sec. or more) a reduction in the intensity of the light lengthens the reaction time. The relation has been dealt with already in Part I and the curves in Fig. 1 show

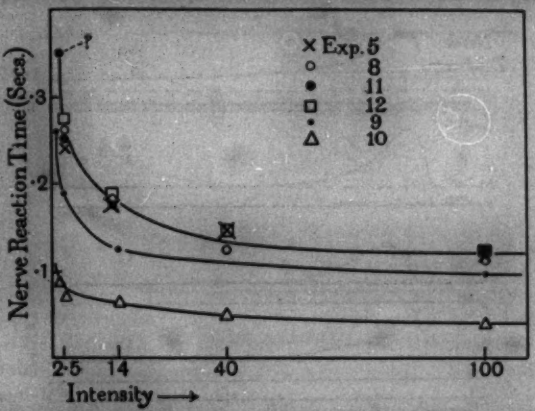

Fig. 1. Relation between intensity of light and nerve reaction time. Duration of exposure >·5 sec. Light 100 = 560 metre candle illumination of disc. Area of disc 1024 mm.², giving retinal image ·95 mm. diam.

its form in six experiments of the present series. It will be seen that the reaction time begins to lengthen rapidly when the illumination of the disc is reduced below about 50 metre candles[1]. Now if we use an illumination giving a fairly short reaction time and try the effect of reducing the duration of the exposure, the reaction time remains unchanged as long as the duration of exposure exceeds a certain critical value determined

[1] There is, however, an upper limit to the reaction time. With the preparations used in these experiments if the light was not strong enough to produce a nerve response in ·35 sec. it was unable to do so at all. In our winter experiments we found reaction times as long as ·6 sec.

by the intensity of the light. With exposures shorter than the critical duration the reaction time is lengthened. The magnitude of the discharge (the total impulse frequency) in its early stages is also unchanged as long as the critical duration is exceeded and the only difference in the form of the response is a somewhat more rapid decline with the shorter exposures. It is not always possible to detect this, for the response to the shortest flash lasts for fully $\frac{1}{4}$ sec., and in $\frac{1}{4}$ sec. the response to continued illumination has fallen a long way from its maximal value. The form of response (*i.e.* the curve showing the total impulse frequency at any time) to steady illumination and to a flash of greater than the critical duration is shown diagrammatically in Fig. 2 *A* and *B*. The

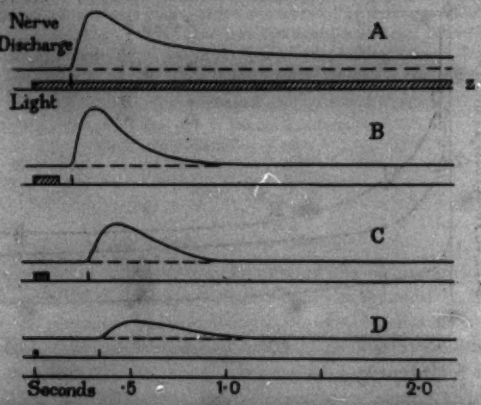

Fig. 2. Diagram, drawn roughly to scale, to show change in magnitude (*i.e.* total impulse frequency) and reaction time of optic nerve discharge as the duration of exposure is reduced.

difference in the curves is very small, for the continued illumination merely prolongs the discharge at a much lower level than that of the initial outburst.

With light 14 the critical duration of the exposure lies between ·03 and ·06 sec. When the flash is shorter than this the reaction time lengthens and the response is smaller. The reduction in the response is mainly a reduction in the maximum impulse frequency, for the duration of the discharge is not appreciably altered. Even with a flash of only ·005 sec. the impulse discharge lasts for $\frac{1}{4}$ sec. or more, though the low frequency

makes it difficult to be sure whether the duration is really unaltered. The changes produced by reducing the duration of the flash below the critical interval are shown in Fig. 2 *C* and *D*.

For a given intensity, then, there is a critical duration which the flash must possess if it is to have the maximum stimulating effect judged both by the shortness of the reaction time and the frequency of the impulses. The change in the effectiveness of the flash when its duration is reduced below the critical value is best examined by measuring the reaction time of the discharge, since this can be determined more accurately than the impulse frequency. The results of a typical experiment are given in Fig. 3. It will be seen that the lengthening of reaction time

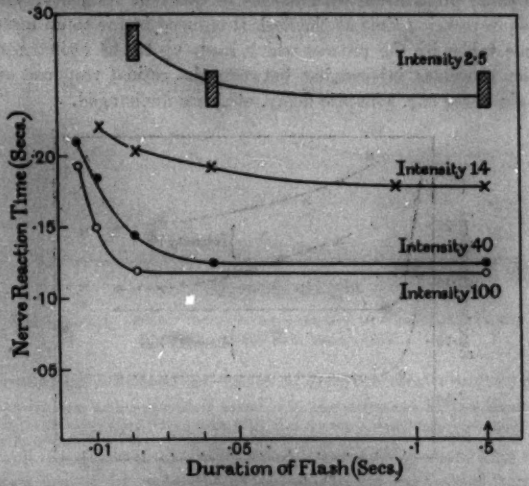

Fig. 3. *Exp.* 8. Nerve reaction time for flashes of different intensity and duration.

with flashes of less than the critical duration is most clearly marked when the intensity of the light is greatest. With low intensities the reaction time, which is long even with continued illumination, rises very slowly as the duration of the flash is reduced and it is difficult to specify the particular duration at which the rise begins. But with these low intensities, although the reaction time does not change much, the magnitude of the response falls off considerably and with the very short exposures it is usually impossible to detect any discharge at all.

Retinal reaction time. We are dealing here with the nerve reaction time, *i.e.* the time taken by the whole sequence of processes which culminate in the activity of the optic nerve. In Part I we pointed out that the nerve discharge is preceded by the development of the initial negative electric response of the retina (the *a* response) and that the retinal-nerve interval remains approximately constant for all intensities of light, *i.e.* for all values of the reaction time. These results were obtained with exposures of long duration, but the present experiments show that the retinal-nerve interval remains constant for flashes also. An example of this is given in Fig. 4, which records the nerve reaction time and the retinal reaction time (time to beginning of *a* response) in the same preparation with flashes of different intensity. Thus the increase in reaction time as the flash is reduced is due to an increase in the time taken by the process which leads up to the retinal response; and the processes intervening between the retinal response and the nerve discharge (*e.g.* synaptic delay, etc.) are unchanged.

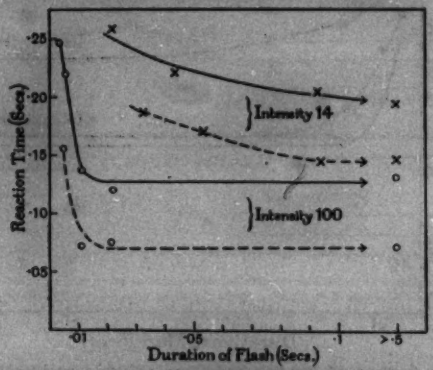

Fig. 4. *Exp.* 13. Retinal reaction times (dotted curve) and nerve reaction times (continuous curve). The retinal-nerve interval remains constant.

The beginning of the nerve discharge is more easily measured than the beginning of the retinal response and most of our experiments have been made on the former. But it must be borne in mind that the nerve reaction times which appear in the curves are divisible into a varying retinal reaction time and a fixed retinal-nerve interval. For the change in the total reaction time to appear in its proper perspective the curves

should all be shifted nearer the base line by an amount equal to the retinal-nerve interval (·05 to ·08 sec. in the present experiments).

Quantity relation. The curves in Fig. 3 do not suggest any simple relation between the reaction time and the duration and intensity of the flash, but if the data are presented in a slightly different form the relation becomes clear enough. In Figs. 5 and 6 the abscissae instead

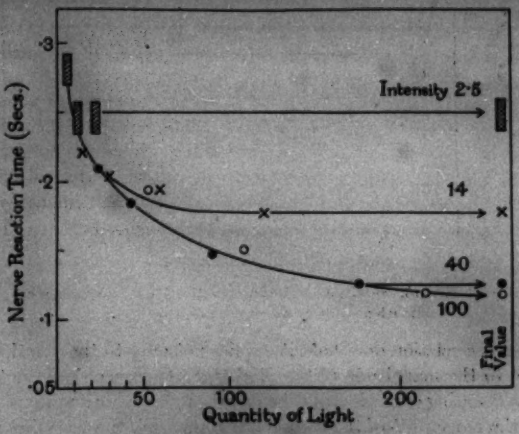

Fig. 5. *Exp.* 8. Relation between nerve reaction time and total quantity of light (intensity × duration × 100).

of representing durations are made to give the total quantity of light received by the eye over unit area[1], *i.e.* the duration of the flash multiplied by the intensity, and the result is that the rising part of the curves all coincide within the limits of experimental error. This relation has been tested in eight preparations and no exceptions have been found. It shows that for short flashes of different intensity the reaction time depends simply on the total quantity of light received by the eye, a bright flash of very short duration giving the same effect as one of less intensity and longer duration. The relation only holds with flashes which are short compared with the reaction time and in any case

[1] The units of quantity in Figs. 5, 6, 8 and 9 are nearly identical with those used later in Figs. 10 and 11, where different areas are exposed. In the present experiments the quantity is given by *intensity* × *duration in sec.* × 100. In Figs. 10 and 11 the quantity for the same area (area 102) is given by *intensity* × *duration in sec.* × 102.

shorter than the critical duration for the particular intensity of light. When they exceed this duration the reaction time settles down to a steady value which is determined by the intensity alone.

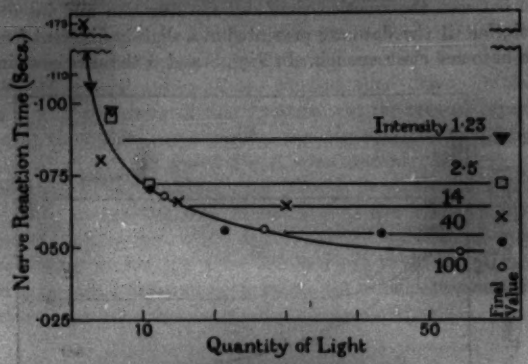

Fig. 6. *Exp.* 10. As Fig. 5. In this preparation the reaction times are much shorter than those usually obtained.

When the relation does hold it applies not only to the reaction time but also to the magnitude of the discharge. Our records do not allow accurate counting of the impulse frequency, but as long as the frequency is high the movement of the mercury shadow from the base line gives a rough idea of the summed effect of the impulses at any moment. Using this criterion it is found that a given reaction time implies a given magnitude of discharge, whether this is caused by a bright flash of short duration or a dim flash of longer duration. Examples of this are given in Fig. 7. In *A* and *B* the quantity of light is 2–3 times as great as in *C*, *D* or *E*. The figure shows also the effects of a change in area (discussed on p. 294) and record *C* is included as an example of the long reaction time produced by a very low intensity.

Comparison with human vision. For short exposures, then, the stimulating effect, judged by the optic nerve discharge, depends simply on the total quantity of light received by the eye, and it is interesting to find that this result agrees with the relation found in man between the quantity of light received in a flash and the brightness of the resulting sensation. McDougall(3) has shown that for a given intensity of light to produce the maximum brightness the flash must have a definite duration, which he calls the "action time." If the duration is less than

Fig. 7. *Exp.* 9. Electrometer records of nerve response to flashes. Time marker gives ·125 sec. intervals. Period of exposure signalled by the shadow at the foot of each record. The white line marks the moment taken as the beginning of the discharge.

In *A* and *B* the size of the retinal image is changed, but the total quantity of light is the same (see p. 294). *C* shows the delay occurring with a very weak intensity where a long exposure is required for a given quantity of light.

A.	Intensity 14	Area 102	(=retinal image ·95 mm. diam.)		Duration = ·021 sec.		
					Quantity ($I \times A \times t$) = 30		
B.	,, 100	,, 6·4	(=retinal image ·24 mm. diam.)		Duration = ·043 sec.		
					Quantity ($I \times A \times t$) = 28		
C.	,, 1·6	,, 102	Duration	·098 sec.	Quantity	16	
D.	,, 2·5	,, 102	,,	·043	,,	11	
E.	,, 14	,, 102	,,	·006	,,	8·6	
F.	,, 100	,, 102	,,	·021	,,	214	

this, the sensation diminishes and depends simply on the total quantity of light (intensity × duration). He points out that the Talbot-Plateau law of flicker necessarily involves such a relation. McDougall's "action time" evidently corresponds with our "critical duration" at which the reaction time and the magnitude of the response becomes constant, and it is satisfactory to know that the same laws hold good for the optic nerve discharge and for the visual sensation.

Nature of retinal changes: Hecht's scheme.

The quantity relation gives some clue to the processes involved in the action of light on the retina. With a brief flash the response begins some time after the light has ceased to act, and it begins sooner or later according to the quantity of light received. We have therefore two processes to consider, a primary change coinciding with the exposure and a slower, secondary change following on the primary change and ultimately producing the response. The primary change produces something which we may call the "light product" or "light effect" and the amount of light product is directly proportional to the quantity of light in the flash. The rate of the secondary change is determined by the amount of light product and when it has progressed to a definite extent the retinal response begins. This is merely a re-statement of the results and involves no assumptions as to the nature of the changes.

A scheme involving two reactions of this kind has been worked out by Hecht(3) for the light response of two invertebrates, *Mya* and *Ciona*. In these animals the light-sensitive organs are much less elaborate than the vertebrate eye and the response to light consists simply in a retraction of the syphons of the animal. Hecht's data are of the same type as ours, i.e. measurements of the reaction time to different stimuli, and the present results are best considered in relation to Hecht's scheme. Hecht concludes that the primary change is a photochemical decomposition of a light-sensitive substance S into the products P and A. The reaction is reversible, $S \rightleftharpoons P + A$, and the recombination occurs in the dark and accounts for the phenomena of dark adaptation. The secondary change is a distinct chemical reaction, $L \rightarrow T$, which is catalysed by the products $P + A$. The evidence for these conclusions will be dealt with in the course of the discussion.

We will take first the primary change. In *Mya* and *Ciona*, as in the eel, the response can be produced by a flash of light and takes place some time after the flash is over. For light of a given intensity the reaction time remains constant until the duration of the flash is reduced

below a critical value, which Hecht calls the sensitisation period, and with shorter flashes the reaction time is lengthened. The sensitisation period varies inversely with the intensity of the flash, the product of the two being constant, and for flashes of the sensitisation period for any given intensity the "latent period" between the end of the flash and the beginning of the response has a constant, minimal value. Thus a constant quantity of light (intensity × sensitisation period) is required to give a constant effect, *i.e.* the shortest possible latent period. In photochemical changes a given quantity of light produces a given amount of change (Roscoe-Bunsen law) and the temperature coefficient of these changes is nearly always very small. Hecht finds that the temperature coefficient of the sensitisation period is also very small and concludes that the primary change is a photochemical reaction[1]. This is supported by the fact that in *Mya* the minimum quantity of light needed to produce a response is also constant and has a temperature coefficient at only 1·06 for 10° C.

Now our results on the eel evidently point to a primary change of the same kind as in *Mya* and *Ciona*, for we find that for short flashes a given quantity of light produces a given length of reaction time and size of discharge. It is known also that for short flashes the minimal quantity of light required for visual sensation in man is approximately constant (cf. Prentice Reeves(4)). The only divergence between our results and Hecht's occurs when we use flashes which are as long as the critical duration or sensitisation period, *i.e.* the duration at which the reaction time becomes constant. The critical duration increases when the intensity is reduced, but the product of the two is not constant and diminishes with low intensities, in other words the quantity of light required to make the reaction time constant is smaller with low intensities than with high. This may be seen from Table II and the same change may be seen from McDougall's figures for the action time in man (the duration for maximum brightness) which are given in Table III.

This discrepancy need not concern us very much. In *Mya* or *Ciona* we must suppose that the secondary reaction does not start until most of the light product has been formed. Thus the rate at which it is formed would have no appreciable influence on the rate of the secondary reaction. In the eel's eye the secondary reaction is much more rapid and we may suppose that it starts as soon as any of the light product is present. With short flashes of high intensity the light product will all

[1] Photoelectric effects are also independent of temperature and directly proportional to the quantity of light.

TABLE II.

Conger vulgaris. Relation between light intensity and "critical duration."

Exp. 5			*Exp.* 6		
Intensity	Critical duration	Product (×100)	Intensity	Critical duration	Product (×100)
100	·015 sec.	150	100	·01 sec.	100
40	·02	80	40	·03	120
14	·03	42	14	·04	56
2·5	·06 (?)	15 (?)	2·5	·10	25
Exp. 8			*Exp.* 9		
100	·02	200	100	·02	200
40	·04	160	40	·025	100
14	·07	98	14	·05	70
2·5	·08 (?)	20 (?)	2·5	·08	20

TABLE III.

(McDougall. Brit. Journ. of Exp. Psychol. I. p. 168. 1904.)

Man. Relation between light intensity and "action time."

Intensity	Action time	Product (×1000)
1	·049	49
$\frac{1}{2}$	·055	27
$\frac{1}{4}$	·061	15
$\frac{1}{8}$	·066	7·6
$\frac{1}{16}$	·10	1·5

be formed before the secondary reaction has had time to progress very far and the conditions will approximate to those in *Mya* and *Ciona*. With low intensities, however, the product will be formed slowly and the rate of formation may be very little faster than the rate of progress of the secondary reaction. The beginning of the retinal response will therefore depend more on the rate of formation of the light product (*i.e.* simply on the intensity of the light) and less on the total quantity of light reaching the eye. An explanation on these lines is needed to account for the change in shape of the curves in Figs. 3 and 4, with different intensities. The flattening with low intensities is evidently related to the more gradual rate of increase of the light product. For the same reason the quantity relation is only obeyed with flashes which are short compared with the reaction time (cf. Fig. 7 *C*).

We have made no measurements of the effects of temperature, but the present evidence is enough to justify the view that the primary change is of the same nature in the vertebrate eye as in *Mya* and *Ciona*. Evidence of another kind has been brought forward by Hecht, for he has shown that the progress of dark adaptation follows the same course in man as in these animals and that it can be explained in terms of the reaction which occurs when the products $P + A$ recombine in the dark to form the sensitive substance S.

We now come to the secondary change and here too we find no reason to depart from Hecht's scheme. In this it is regarded as a separate reaction, $L \rightarrow T$, which is catalysed by the light and causes the response when a sufficient amount of T has accumulated. The temperature coefficient of the latent period in *Mya* is high and the quantitative behaviour in relation to temperature agrees with that of a chemical reaction. But the most important fact in deciding the concrete form of the hypothesis is the relation between the quantity of light and the rate of the secondary change. The rate of change is given by the reciprocal of the latent period and in *Mya* this is a linear function of the quantity of light in the flash. This rules out the possibility that the secondary change is a diffusion of the light product from the place where it is formed to the place where it produces the response, for the rate of diffusion is only a linear function of the concentration when the diffusion proceeds into a large volume where the concentration may be regarded as zero. The hypothesis of a chemical reaction involving the destruction of the light product leads to various complications, and the catalysis of a separate reaction offers the simplest explanation of the data. As Hecht is careful to point out, we need not accept this as more than a working hypothesis, but we can see whether it applies in the eel's eye as well as to *Mya* by finding out whether the linear relation is or is not obeyed in the eel.

In Figs. 8 and 9 the dotted curves show the relation between the quantity of light and the reciprocal of the nerve reaction time. The curves are not straight and there is no reason why they should be, for the process whose rate we are considering must be complete at the moment when the nervous mechanism of the retina is excited and this will occur some time before the discharge in the optic nerve. The retinal electric response precedes the nerve discharge by a fixed interval and so is closer to the beginning of the retinal excitation. Thus, if we take the reciprocal of the retinal reaction times we shall obtain values which are much more nearly proportional to the rate of the secondary reaction. In the two experiments shown in Figs. 8 and 9 several measurements of the retinal-nerve interval were made with various lights and the average was found to be ·08 sec. in Exp. 8 (Fig. 8) and ·065 sec. in Exp. 11 (Fig. 9). Subtracting these intervals from the nerve reaction times gives the retinal reaction times, and the reciprocals of these are plotted in the figures by the upper set of points joined by the continuous line.

In Fig. 9 (Exp. 11) the points conform very closely to a straight line

for quantities of light varying from 5 to 50 units. Fig. 8 (Exp. 8) is drawn on a smaller scale to show the effect of larger quantities. Up to

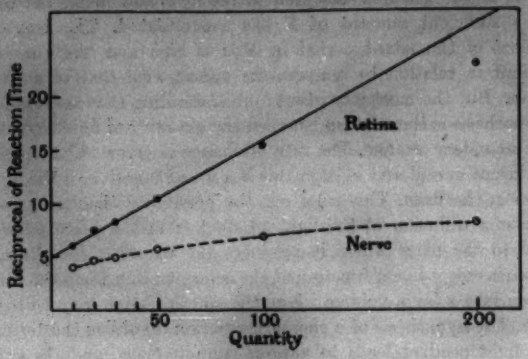

Fig. 8. *Exp.* 8.

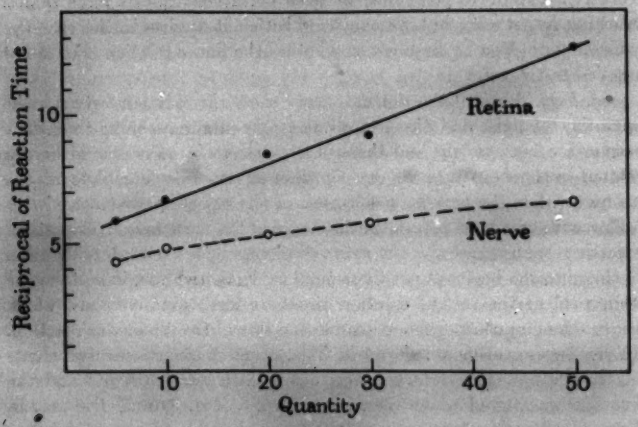

Fig. 9. *Exp.* 11.

50 units the divergence from a straight line is scarcely appreciable, but at 100 units the point falls below the line. The divergence is still greater at 200 units. The same divergence with large quantities appears in Exp. 11

and Exp. 13 (the only other experiment in which we have the necessary data). This divergence is only to be expected, for our figures relating quantity with reaction time cannot really be applied over such a range. To give 100 units our strongest light must last for ·01 sec. and such a duration is very near the critical value for this light. Thus an increase to 200 units (·02 sec.) causes very little change in the reaction time or its reciprocal, and a further increase in the duration of the flash would produce no change at all. The "quantity relation" is only true for flashes which are appreciably shorter than the critical duration, and over this range the points in Figs. 8 and 9 lie on a straight line[1].

In the other experiments the retinal-nerve interval was not measured, but if we assume the linear relation and then calculate the interval we obtain values which range between ·06 and ·09 sec., *i.e.* values which agree well enough with the measured intervals in Exps. 8, 11 and 13. The only exception is Exp. 10 (shown in Fig. 6) where the interval works out at ·04 sec., but in this preparation the nerve reaction times are so short that the interval cannot in any case be longer than ·045 sec.

As a first approximation, then, we may say that for flashes of short duration there is a linear relation between the reciprocal of the retinal reaction time and the quantity of light in the flash. This means that the velocity of the secondary change is a linear function of the amount of light product formed, and it is to be noted that we are now using "secondary change" to mean the whole sequence of events which start with the formation of the light product and culminate in the negative response of the retina. Since the duration of the process is so simply related to the quantity of light, the whole of the time is probably taken up by a single reaction, and the beginning of the retinal response must follow without delay as soon as the reaction has progressed to a definite extent.

In all our experiments the reciprocal curve (whether for nerve or retina), if prolonged backwards, cuts the y axis some way above the origin. The interpretation of this is not very clear, but the result is interesting because it diverges from Hecht's. His most complete curve passes through the origin and others cut the x axis. In some of our records with very small quantities of light there are faint indications of a very small response occurring after a very long reaction time. We are

[1] The individual variation of the different points may seem suspiciously small, but it must be remembered that the reaction times are taken from the smoothed quantity-reaction time curve and not from the single observations which may lie slightly above or below the curve.

not satisfied that these are anything more than the random irregularities which are generally present in a record from an optic nerve with the eye in darkness, but, if they are true responses, their reaction time shows that the reciprocal curve must bend down sharply as it approaches the origin. If so, there is a lower as well as an upper limit to the quantity of light which gives the linear relation, and we must take this into account in deciding the nature of the secondary reaction.

For the present we may conclude that within certain limits the rate of the secondary reaction in the eel's eye, as in *Mya*, is a linear function of the quantity of light, so that Hecht's arguments will apply equally well in either case. But we have still to deal with another factor which must be taken into account in deciding the nature of the changes. This is discussed in the following section.

Relation between area and intensity.

In our previous paper we investigated the effect of altering the size of the retinal image. With retinal images up to 1 mm. diam. we found that an increase in size had the same effect as an increase in the intensity of the light—the maximum frequency of the impulses increased, but not in direct proportion to the area, and the reaction time became shorter. Both the reaction time and the frequency seemed to depend approximately on the total quantity of light (area × intensity) reaching the eye in unit time without regard to its distribution. We pointed out that these results agree in the main with the relation between threshold intensity and area in man, for here too, if the image is small (·5 mm. diam. or less), an increase in size can compensate for a decrease in the intensity of the light.

In our previous work we used continuous illumination. This complicates the issue to some extent and we have now extended these results by using brief flashes in which the intensity, duration and area illuminated are all variable. The area was controlled by altering the size of the illuminated disc. The image was focussed on the retina by the necessary lenses and tests with small windows cut in the sclerotic showed that a sharp image was formed.

The result is quite definite and may be seen at once from Figs. 10 and 11. In these the ordinates are the nerve reaction times and the abscissæ the total quantity of light (intensity × duration × area). The retinal areas have been varied from ·084 to ·95 mm. diam. and we have given a rough indication of the size of the image by drawing circles of corresponding size to mark the points. It is clear that the points are

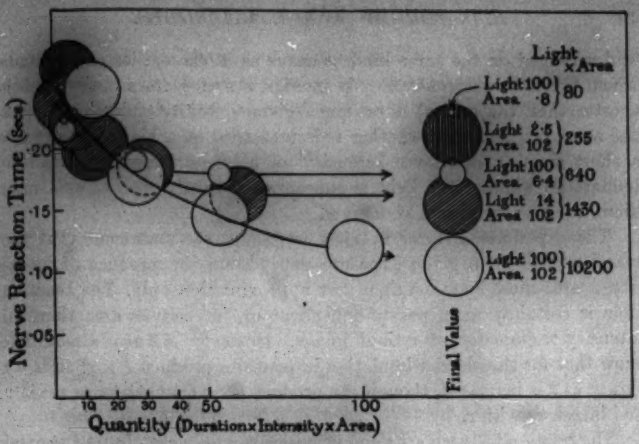

Fig. 10. *Exp.* 11.

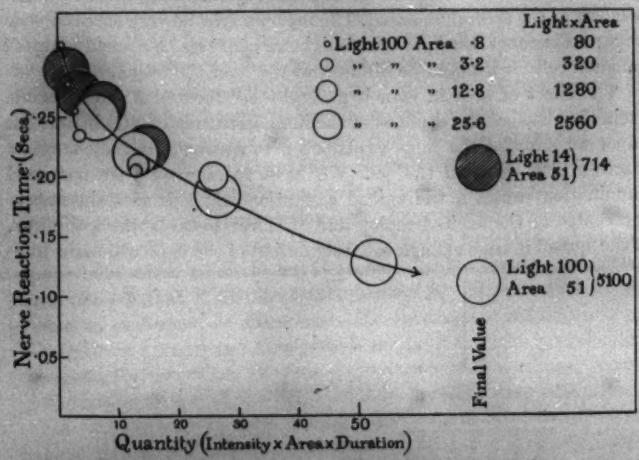

Fig. 11. *Exp.* 12. Quantity-reaction time curves showing effect of change in area as well as in intensity and duration. Areas given in arbitrary units (actually $\frac{1}{10}$ area of illuminated disc).

Area 102 corresponds to a retinal image ·95 mm. diam.

"	51	"	"	·57	"
"	25·6	"	"	·33	"
"	6·4	"	"	·23	"
"	3·2	"	"	·17	"
"	·8	"	"	·084	"

still arranged on the same kind of curve as in the earlier experiments where the area was constant. As in Figs. 5 and 6, for a flash which is shorter than the critical duration, a given quantity of light produces the same reaction time whether it is presented at a high intensity for a short time or at a lower intensity for a longer time, and it is also immaterial whether the light is concentrated over a disc of ·084 mm. diam. or spread over one of ·95 mm.

The experimental error is fairly large and the statement that the reaction time for a given exposure depends on the product of intensity × area must be taken as a first approximation only. The reaction time is certainly more nearly dependent on intensity × area than on intensity × diameter of retinal image. However, Abney's results(5) show that for threshold stimulation in man the product $I \times r^2$ falls off slowly as r is increased, though the product $I \times r$ changes more rapidly and increases with r.

The relation between area and intensity can only mean that the total effect of the light is transmitted to some region whose extent is independent of the area illuminated. The region might be very small or very large; for instance, the light effect might all converge to a small group of ganglion cells or it might be diffused widely over the retina.

The effect of area in man is generally ascribed to a spread of the excitation beyond the confines of the illuminated region and in our first paper we adopted the same explanation to account for the change in reaction time, etc., in the eel. We have to suppose that restricted illumination causes a widespread excitation which is most intense in the centre of the excited region and declines towards the periphery. If the spread is large enough the distribution of the light will make little

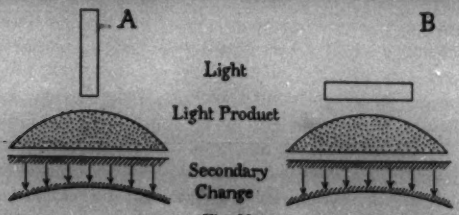

Fig. 12.

difference to the distribution of the excitation and the intensity of excitation at the centre will depend approximately on the total quantity of light which reaches the eye. Fig. 12 may make this clearer.

The second possibility is represented in Fig. 13, where a large area of the retina is connected to a single ganglion cell and the effect of the light is concentrated instead of diffused.

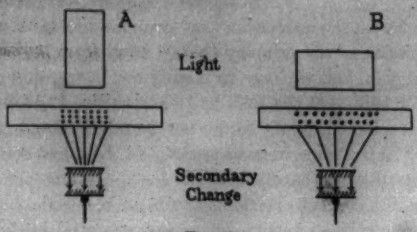

Fig. 13.

Whichever scheme is chosen it is clear that the process of diffusion or concentration must take place very rapidly, so rapidly that it has no appreciable influence on the reaction time; what is still more significant is that the process must take place during the primary change. In Figs. 12 and 13 the lapse of time during the secondary change is indicated by the small arrows. As the integration of the light effect is assumed to take place before the secondary change begins, the distribution of the light will make no difference to the rate of the reaction. It is much more difficult to explain our results on the view that the integration occurs during or after the secondary change. If the latter takes place in a region coinciding with the illuminated area it will go on more rapidly when the light is intense and the reaction time must therefore be shorter in case *A* (Figs. 12 and 13) than in case *B*. On the hypothesis of a spread of the light effect we might try to account for the equal reaction times by supposing that a definite total quantity of the exciting substance *T* has to be formed in either case, the substance spreading out very rapidly from a restricted area just as the light product is supposed to spread in Fig. 12. Since the rate of formation of *T* is a linear function of the concentration of the light product, it might seem that the time taken to form a given total quantity of *T* would be the same with a light of intensity $2I$ acting on an area a as for one of intensity I acting on an area $2a$. But this would only be true if the rate of formation of *T* were directly proportional to the quantity of light which falls on a given area. The curves in Figs. 8 and 9 show that this is not the case. Doubling the quantity of light does not halve the reaction time, for all the curves cut the ordinate axis some way above the origin. Thus a light

of $2I$ in an area a would take longer to form a given quantity of T than a light I on an area $2a$. Various subsidiary assumptions might get over this difficulty, but none of them seems likely enough to be worth following up.

We are left with the conclusion that there is an instantaneous, or very rapid transmission of the primary change away from the confines of the illuminated region, and we have to modify our conception of the change to account for this. On the first hypothesis (Fig. 12) a mere diffusion outwards of the light product would not be rapid enough, for the effect must travel at least ·2 mm. in less than ·002 sec.[1], and an actual scattering of the light itself could hardly account for so wide a spread. The most likely explanation seems to be that the spread is electrical, that the light forms something which causes a change of potential in the illuminated region and the light product is the electric field set up in and around the region. We do not propose to discuss this at length as we have no fresh evidence to present. Fröhlich(6) has shown that the potential changes which make up the normal retinal response are not confined to the illuminated region, but this does not help our problem, for these changes do not appear until the excitation of the retina has already taken place. So far as we are aware there is no evidence of an immediate difference of potential between an illuminated and a non-illuminated area.

The chief difficulty with the hypothesis of a spreading excitation is that it gives no clue to the mechanism of form perception in the human eye. It is unlikely that the processes involved in the excitation of the cones can differ fundamentally from that of the rods, yet it is hard to believe that the excitation of the cones does not coincide accurately with the area illuminated. The hypothesis of a concentration of the light effect (Fig. 13) does not raise the same difficulty, for here the excitation of the retinal elements is confined to the illuminated area and the amount of concentration will depend on the nervous connections. This hypothesis (which arises from a suggestion made to us by Prof. Hecht) is represented in greater detail in Fig. 14. Suppose that there are three adjacent retinal areas X, Y and Z which are all supplied by the same three nerve fibres, and that each area contains a large number of elements of varying excitability distributed so that the number excited is proportional to the intensity of the light. Then the total number of excited elements in all

[1] In any diffusion phenomenon where k is the diffusion constant, t the time and d the distance in cm. the quantity kt/d^2 must be of the order of 1 or 2 if appreciable diffusion is to have gone on. Even for nitric acid k is of the order of 2×10^{-5}, so that if $d = ·02$ cm., t has to be of the order of minutes.

three areas will vary with the quantity of light (intensity × area) and the number of excited elements connected to each nerve fibre will vary in the same way. If we suppose that the reaction time of the retinal response depends on the number of excited elements converging on some part of the chain of neurones (*N*, Fig. 14), the changes in reaction time with intensity and area are readily explained. On this view the primary "light product" may be regarded as the excitation of the retinal elements. This must take place without delay as soon as the photochemical reaction has produced enough of the exciting substance, and the excitation must be transmitted rapidly to the point *N* where the different elements converge. The secondary change is the reaction occurring at this point, a reaction which proceeds at a rate proportional to the number of excited elements and leads ultimately to the retinal response and the discharge of impulses in the optic nerve fibres. The transmission of the excitation must be something akin to nervous transmission and the reaction at *N* must be something akin to nervous summation, but we need not assume that the retinal elements transmit impulses of the usual type, and we need not assume that the secondary reaction is something radically different from that postulated in Hecht's scheme. There is certainly no reason to doubt the possibility of overlapping in the areas of distribution of the optic nerve fibres. Even assuming that no overlap occurs we found that one fibre of the eel's optic nerve must supply an area of at least ·08 mm. diam. on the retina and it is therefore quite possible that it may be actually in touch with an area as great as 1 mm.

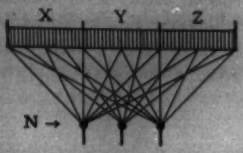

Fig. 14.

It is to be noted that on this view the primary and the secondary reaction take place in different places and the two are linked by a nervous mechanism. The initial electric response of the retina must indicate the activity of the deeper parts (*e.g.* the ganglion cells) and not that of the rod and cone layer. In other respects there is little to distinguish this hypothesis from that of the spread of excitation. We have no evidence which will allow us to decide between these alternative views. Either would have important consequences, but for the present the complexity of the vertebrate retina has prevented us from making any crucial experiment. In the end it may be necessary to seek for this in some of the simpler types of eye. Meanwhile, however, we may still accept Hecht's scheme as a working model for the vertebrate eye as well as for *Mya* and

Ciona. The only alteration we have to make concerns the relation between the primary and secondary changes. This can scarcely be the simple catalysis of the secondary reaction by the light product, for whichever view we adopt it seems clear that the secondary reaction takes place in a region which does not correspond strictly with the illuminated area.

Summary.

The electric response of the retina and of the optic nerve of *Conger vulgaris* to flashes of light have been studied by measurements of the reaction time and of the form of the response. The reaction time is defined as the time between the beginning of the flash and the beginning of the response. The following results have been obtained.

1. For a given intensity of light, flashes which exceed a certain critical duration all give the same reaction time and a discharge of the same maximum impulse frequency in the optic nerve. With shorter durations the reaction time is longer and the impulse frequency is smaller.

2. Using flashes which are somewhat shorter than the critical duration it is found that a definite quantity of light (intensity × duration) over a given area always produces a definite reaction time and a response of definite impulse frequency whatever the intensity of the light.

3. In these circumstances the reciprocal of the retinal reaction time (*i.e.* the rate of the process which determines it) is a linear function of the quantity of light over a considerable range of quantities.

4. These results hold good not only for changes in the intensity and duration of the flash but also for changes in the area illuminated. A definite quantity of light (intensity × duration × area) produces the same reaction time though the retinal area illuminated varies from ·08 to ·9 mm. diam.

5. The processes leading to excitation may be divided into a primary change coinciding with the flash and forming a product proportional to the quantity of light received, and a secondary change outlasting the flash and leading ultimately to the excitation of the nervous structures. The rate of the secondary change is a linear function of the amount of "light product" or "light effect" formed in the primary change.

6. The relation between area and intensity must mean that the total effect of the light is transmitted to some region whose extent is independent of the area illuminated. The effect might be diffused widely over the retina or concentrated into a small number of nervous paths which have an overlapping distribution on the retinal surface.

7. In either case the transmission of the light effect must take place rapidly as part of the primary change and the secondary change must occur in the region to which the light effect is transmitted.

8. These changes are discussed on the basis of Hecht's scheme for the light reactions of *Mya* and *Ciona*. The results are found to agree in the main with Hecht's and the only important modification which must be made for the eel's eye lies in the relation between the primary and the secondary change.

We have to thank Dr R. W. Gerard for his valuable help in making and interpreting these experiments and to thank the Government Grants Committee of the Royal Society for a grant towards the expenses involved.

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A DISCUSSION ON THE PART PLAYED BY THE SUPRAVESTIBULAR CONNECTIONS IN DECEREBRATE RIGIDITY.

By Dr L. J. J. MUSKENS (*Amsterdam*).

During the past few years I have had but limited laboratory facilities for performing experimental operations connected with the remarkable phenomenon of decerebrate rigidity, but I have succeeded in carrying out a number of experimental lesions in cats involving the region of the posterior commissure.

The cases here recorded are only those where the animal survived for a sufficiently long period to make the subsequent examination of the nervous tissue with the osmic acid stain practical.

I consider it important to distinguish those cases which present complete immobility with opisthotonos as described by Sherrington, Horsley and Loewenthal from those with increased tonicity on one side, the power of locomotion being preserved (1).

It is not surprising to anyone who has made a study of the effect of lesions in the posterior longitudinal fascicle and commissural nuclei to find forced movements closely associated with decerebrate rigidity. Further, because symmetrical lesions are very rare in this region phenomena related to forced movements are bound to occur. Sherrington has all along described conditions, either of stimulation or of extirpation, which alter the condition of decerebrate rigidity or give it a special character.

The original writers on the subject denied the existence of cerebellar influences on the phenomenon of decerebrate rigidity, but it must be admitted that later work has demonstrated that the anterior cerebellar lobe does exert some influence. Further, different writers are not in agreement in their descriptions of the effect of lesion and stimulation of the crus anterior cerebelli and Gowers' tract. The fact, however, seems to be established that ablation of Deiters' nucleus, hemisection of the medulla oblongata, section of the antero-lateral tract of the cord and also of the posterior roots does away with the rigidity when it has once appeared.

Thiele, Weed, Warner and Olmsted demonstrated that unilateral section of the truncus cerebri and the rhinencephalon produced a certain

degree of rigidity, certainly at least a temporary lateral increase of muscle tone. It is curious to note that if this unilateral section is made above (*i.e.* anterior to) the posterior commissure the resulting stiffness is heterolateral, while if the section be caudal to the commissure the stiffness is homolateral.

In the first observations on decerebrate rigidity it was noticed that a special posture was associated with the condition, the neck was bent, the concavity being directed backwards. Sherrington recognised in a complete demonstration of decerebrate rigidity a "physiological entity." Such a posture is a forced position in the vertical plane such as I described as occurring after certain lesions involving the primary and secondary vestibular connections (2). Although Sherrington speaks of a special posture Magnus speaks of a "caricaturé of a posture," because the physiological movement upward is exaggerated. If, however, the dictum of Liddell and Sherrington is recalled which states "Reflex standing is a composite postural reflex the anti-gravity muscles counteracting the superincumbent weight," (3) it is justifiable to expect *a priori* that the mesencephalic structures which govern these anti-gravity muscles may play an important part when the interruption of certain inhibiting tracts sets the action of these structures free.

Here it is worth while to recall a forgotten observation of Ewald to the effect that a pigeon blindfolded and with both labyrinths removed takes the extreme position of "culbutation" backwards, neck and eyes turned maximally overhead. Again certain observations in the pigeon tend to prove, that upward movement of the eyes is dependent on an entirely different mechanism, compared with lateral and downward movement, and might be considered as an effect of release from various innervations. Also it is well known, that in man during sleep the eyeballs are directed upwards.

The following points appear to me to require consideration. Warner and Olmsted concluded that the spino-cerebellar and Gowers' tracts supplied the efferent proprioceptive impulses for the phenomenon of decerebrate rigidity, hence stimulation of these tracts would alter the fundamental conditions of the phenomenon.

It must be recalled that Marburg and Bing showed that lesions of these tracts were followed by forced movements in the vertical plane and a tendency to fall backwards. On the other hand, Warner and Olmsted concluded that the efferent impulses necessary for decerebrate rigidity arose in the neighbourhood of the red nuclei but not in the red nuclei themselves.

In birds, although a true condition of decerebrate rigidity could not be induced, Martin Rich observed quite accurately in birds that forced movements in the horizontal plane on one side associated with a tendency to fall to the other side invariably followed after cross-section of the mid-brain.

Weed noted that hæmorrhage in the raphe of the truncus cerebri may cause flexion decerebrate rigidity instead of the exaggerated posture of the extensors.

Magnus and de Kleijn have shown that the condition of decerebrate rigidity varied considerably with the position of the animal at the time.

Decerebrate rigidity has always been regarded as a release phenomenon ("Enthemmung") in which severance of the prosencephalon from the mesencephalon was held to be the essential feature. Although this was the general belief yet it was realised that the proof was unsatisfactory.

Ken Kure recently propounded the view that the occurrence of hæmorrhage was one of the conditions necessary for the exhibition of decerebrate rigidity. From my own observations it seemed to me very probable that some special alteration in the condition of the mid-brain such as hæmorrhage would induce is necessary for the demonstration of complete decerebrate rigidity. On the other hand, the results of observations by Bazett, Penfield and Rademaker seem to exclude the possibility of such a complication being a main factor in bringing about the condition.

In the thirty years which have elapsed since decerebrate rigidity was first recognised two main lines of investigation have been followed. In the first and earlier work attention was directed to the different influences affecting decerebrate rigidity. In the later work, started by Weed and completed by Rademaker, attention was concentrated on the part played by the red nucleus in the occurrence of this phenomenon. It is remarkable that the second and later group of workers dealt with the subject primarily as physiologists, the anatomical considerations coming second. The examination of all records of the work done on the subject shows no careful analysis of the forced movements in three planes, while no direct application of the latest anatomical knowledge concerning the supravestibular nuclei is attempted.

This curious incompleteness and one-sidedness of the investigation in my opinion accounts for the doubt and uncertainty found in all the literature dealing with decerebrate rigidity.

Before discussing the effect of lesions of the commissural nuclei and their efferent tracts, or attempting to answer the question as to the rôle played by a lesion involving the ascending and descending tertiary vestibular connections in the occurrence of decerebrate rigidity, certain anatomical-physiological data must be recalled. As a result of the work of Cajal, who first suggested the possibility, it was definitely established in 1907 that vestibular nerve fibres pass right into the nucleus tecti cerebelli without any cell stations. This was confirmed later by Villaverde and W. F. Allen(4). Wallenberg and Deqanells observed, in birds, vestibular fibres passing to the lateral nucleus, whereas Trendlenburg, Marx and Frenhel contradicted this statement. Since this date (1907) I have repeatedly directed attention to the fact that a lesion of this roof-nucleus always brings about forced movements in the vertical plane, characterised especially by a tendency to fall backwards with the head and eyes directed upwards. In birds culbutation backwards is peculiarly well marked. Occasionally in the first few days after the lesion has been performed, when compensation is not yet effective, forced movement in the opposite direction is seen, the animal falling forward or displaying forward culbutation. In the case of forced movement in the vertical plane backwards and culbutation backwards I noticed an important point of difference from contrary-wise directed forced movements in the vertical plane—falling forward or culbutation forward—and also from the other types of forced movements in the horizontal and frontal planes (these later types of forced movement I have already dealt with in earlier papers, *loc. cit.*). It is this, forced movement backwards is always associated with an increase in muscle tone, especially of the extensors. This may be due to the fact that the direction of the movement is against gravity while the forced movement in the opposite or forward direction is with gravity. Further, forced movements in the forward direction appear to be associated with a curious tendency to sleep.

It must be clearly realised that the centres and muscles involved in these two forms of forced movements in the vertical plane are not and could not be mirrors (Spiegelbild) one of the other, as is the case with circus movements and rolling movements. In the case of circus movements the locomotion towards the right is really the complete "mirror" of the movement towards the left. In the case of rolling movements to either side the same holds true.

In discussing the cause of the increased muscle tone found in forced movements backwards, whether these movements are brought about by

a lesion of the fastigial nucleus (nucleus tecti) or of its hypothalamic connections[1], it must be recalled that in a series of experimental hemisections a temporary stiffness was found heterolaterally if the lesion were anterior to the posterior commissure or homolaterally if posterior to it. Further, Graham Brown found that when the cross-section of the mid-brain of a monkey is stimulated electrically in front of the posterior commissure, conjugate deviation of the head and eyes to the unaffected side resulted. The earlier work of Knoll and Topolanski, on the other hand, proved that such stimulation applied behind the posterior commissure produced conjugate deviation of head and eyes to the affected side. Further it is known that in a series of hemisections of the brain stem from the region of Deiters' nucleus upwards the forced movements in the horizontal and frontal planes change in direction as soon as the posterior commissure is reached(2).

Is it possible with the available data to approach the subject of decerebrate rigidity and discuss it profitably?

In such a discussion certain errors made by earlier workers can at least be avoided. For example, the fact that the red nucleus and the bundle of Monakow, both prominent neural entities, showed marked degeneration in cases of decerebrate rigidity, led to the conclusion that the red nucleus must be the principal factor in this phenomenon. Not only does the case I shall proceed to describe oppose such a view but the work of Bazett and Penfield and Thiele also oppose such a conclusion. It must at the same time be admitted that as yet no cases of genuine decerebrate rigidity have been examined where the bundle of Monakow has entirely escaped degeneration. Why such a case is unlikely to occur has been suggested earlier in this paper. Finally in reptilians where the red nucleus is but very slightly developed, cross-section of the mid-brain

Fig. 1.

[1] F. Bremer. *Arch. internat. de Physiol.* 19. p. 192. 1922. These researches have gone far to remove any doubt as to the presence of increased muscle tone after lesion of the fastigial nucleus. The possibility that this phenomenon of increased muscle tone is not so much associated with any particular centre as with the movement itself evidently did not occur to the writer.

produces a peculiar condition(5) very comparable with decerebrate rigidity in higher mammals (Fig. 1)(6).

From a general consideration of the evidence it is obvious that attention must be concentrated on the work of von Gehuchten, Cajal, de Lange and Castaldi who agreed in the view that a set of nuclei exist about the posterior commissure which have been proved to be of peculiar significance in relation to forced movements, and which are now recognised to be relay centres for the ascending secondary vestibular connections (refer to the work of Vogt(7) and Riese(8)). So far as is at present known about these nuclei it would appear that they are connected with a large number of ascending vestibular fibres but give rise to a very much smaller number of descending fibres. These descending fibres are situated in the innermost part of the posterior longitudinal bundle and are probably possessed of a functional significance far superior to their anatomical size. As is known, even the most primitive type of vertebrates possess these cells and fibres and in some a particular gigantic nerve fibre—Mauthnerz's fibre—does possess a very important function in relation to locomotion. Van Gehuchten originally concluded from observations made in fishes and on the embryonic stages in higher animals, that the whole of the posterior longitudinal bundle was composed of such descending fibres. Further it is known that the ascending and descending fibres of this bundle are the first to be provided with a medullary sheath.

Rademaker of the Utrecht school has all along been one of the most zealous supporters of the theory that the red nucleus does play an important part in decerebrate rigidity. His statement that a mid-ventral incision in the base of the mid-brain 2½ mm. in depth does not induce decerebrate rigidity, while a similar incision 3½ mm. in depth does is very important. At the same time I am inclined to consider the conclusion drawn by Rademaker not completely justified. It is a well known anatomical fact that the nucleus interstitialis lies in front of the anterior pole of the red nucleus, but these two nuclei are not separate and distinct entities but merge one into the other. It is therefore impossible to localise a lesion explicitly to the red nucleus in the way Rademaker suggests. Further, unless the animal has lived six or seven days after such an experiment as that performed by Rademaker, it is impossible to ascertain by the osmic acid method whether the red nucleus has or has not escaped. One of his own cases (called "Black and white") might be brought forward to support an argument that both the red nucleus and nucleus interstitialis play a part in decerebrate rigidity, but the objection does exist that this animal did not live long enough after the operation, and the rigidity was not classical in type. Further, in Rademaker's experiments the preliminary operation alone produced as a rule forced movements, this in itself rather invalidates conclusions drawn from the second operation. This is more important because at the site of the preliminary operation the secondary vestibular connections finish and the tertiary begin, so that each lesion has in all probability a profound effect on both secondary and tertiary structures. The experiments described by Rademaker, where traction was made, on a thread fixed medially are interesting, but unfortunately

the subject of experiment (*A A*) was killed directly and no microscopic examination of the brain tissue was made. In this case it can be supposed that the supra-vestibular connections associated with vertical forced movement escaped. In the other two cases ("Grisette" and "Kleine Zwarte") Meynert's decussation was certainly severed while there was no degeneration of Monakow's bundle, but the cases were not typical examples of decerebrate degeneration. In one case (Grisette) the tr. interstitio-spinalis or Boyce's tract was degenerated. This tract has curiously enough been called the tractus dorsalis. In two other cases (B. T. and B. R.) a permanent posture in the form of rotation round the axis existed, yet in these cases the correcting reflexes of the labyrinth were present. This encourages me to think that my criterion for a possible lesion in the posterior longitudinal bundle (tendency to forced movement) is a better one, and gives a more exact test than the presence or absence of the righting reflexes. Where **Rademaker** criticises my experiments and maintains that other lesions than those affecting the posterior longitudinal bundle may cause forced movements, he merely adduces the fact that hemianoptic animals may deviate in the horizontal plane. This is sometimes the case with pigeons, but it passes quickly and it is not valid to compare a pigeon with cats and rabbits, the animals used in my research(9). **Munzer** and **Wiener** have shown the preponderance of optic impressions and the number of optic nerve fibres in pigeons greatly exceeds the number of all the other sensory fibres.

Further, in **Rademaker's** recent work on the anatomical exploration of the mid-brain the sharp differentiation of the different forms of forced movement is inadequately dealt with. In animals which survived for a sufficiently long period care was not taken to prepare sections stained by the Marchi method for demonstration purposes, neither were the microscopic preparations so prepared as to indicate clearly the left and right sides[1]. The same objections apply to the stimulation experiments of **Graham Brown**(10). In this case the red nucleus was stimulated and the effect on an existing condition of decerebrate rigidity noted. Here again the proximity of commissural nuclei excludes the possibility of a definite decision.

Langworthy studied the condition of decerebrate rigidity in very young animals as well as in some of the lower types. He conceives of the act of walking as the result of two component factors. One is postural, a contraction of the extensor muscles overcoming the force of gravity and enabling the animal to stand, the other is progressive, and is achieved by rhythmic flexion and extension of the legs. Although the most important component—the act of falling forward—is omitted yet an important contribution is made to the subject. In the young opossum cross-section of the mesencephalon is followed first by prominent progressive movements which later in the life of the animal are replaced by a typical condition of decerebrate rigidity. It is interesting to find that **Langworthy** calls the forced movement backwards which follows cross-section of the secondary (crus anterior cerebelli) and tertiary (resection of both nuclei caudati) vestibular connections an "accentuation of the postural reflex."

From a general consideration of the whole problem it seems impossible to hold any profitable discussion on the phenomenon of decerebrate rigidity without a true appreciation of the tracts and structures associated with the forced movements, more especially those in the vertical plane. For example, how fallacious would the conclusions be drawn from

[1] In order to distinguish the right and left sides in microscopical preparations it has been my custom for many years to make a small incision with a razor on the right side of the basal surface of the brain stem and cord, as soon as the hardening with Müller's fluid has taken place, to make perfectly certain the horse hair used to keep the brain sections apart pierces the sections only on the right side of the median line.

mesencephalic experiments if no account were taken of the known facts concerning the direction of circus and rolling movements. The case described by Spiegel and Nikishawa(11) illustrates this point. Case 9 was an animal where the posterior longitudinal bundle had been severed on the left side. The animal performed, quite in accordance with my observations, circus movements to the right. The authors now state that hemisection in front of the red nucleus causes increased unilateral tonicity of the muscles of the trunk and fore paws and that this induces circus movements to the right. Such apparently contradictory results are resolved when the effect of lesions of the posterior longitudinal bundle are considered.

To establish the rôle played by the commissural supra-vestibular nuclei in the causation of decerebrate rigidity attention must be drawn to the following points:

1. Complete decerebrate rigidity ensues only if vestibular connections, lesion of which causes vertical forced movements, are severed on both sides.

In my case, No. 242, which exhibited classical decerebrate rigidity, there was no lesion of either pyramidal tracts. After this experiment it is superfluous to admit influence from the cerebral hemispheres[1].

2. The complete reversal of the physiological effect at the level of the posterior commissure must not be forgotten. This is well demonstrated when a series of oral to caudal hemisections are made across the brain stem of a quadruped and the condition and localisation of partial and temporary rigidity noted, both on the side of the lesion and on the opposite side.

3. This reversal is also seen where in different animals the posterior longitudinal bundle is severed on one side. Caudal to the posterior commissure the ensuing circus movements are directed heterolaterally, rolling however to the affected side, while the direction of both is reversed as soon as the posterior commissure is reached (2).

4. Graham Brown found the same complete physiological reversal when oral caudal sections were made through the brain in primates when the area of the posterior longitudinal bundle was stimulated.

From a consideration of these data it is evident that section through the secondary and tertiary vestibular connections exerts a very definite influence on the occurrence of decerebrate rigidity. Especially a certain influence must be admitted by those tracts and structures, injury of which results in forced movements in the vertical plane. A certain influence may be ascribed to the red nucleus, but as yet proof of this assumption is not forthcoming.

[1] The animal survived the lesion five days. There were no signs of degeneration in the pyramidal fibres, either ascending or descending, neither were the pedunculi cerebri injured.

With all deference to the work of Luciani, Horsley and Clark and many others, very little is as yet clearly understood about cerebellar localisation and co-ordination. It is known that even a superficial lesion of the cortex diminishes the faradic irritability of the contralateral motor cortex (Rossi(12), Bikeles, Muskens(13)).

Further, it is known that decerebrate rigidity is inhibited by stimulation of the palæo-cerebellum. It may even be admitted that the cerebellum has at least an influence on tonic-postural phenomena (Rossi and Simonelli(14)). In further study of the nature of cerebellar influence it will be well to distinguish the essentially vestibular function of the fastigial nuclei from the purely cerebellar function of the dentate nucleus. The cortex of the mid-lobe might conceivably serve as a relay station for both. To analyse the influence of the brachium anterior cerebelli, and the cerebellum as a whole on decerebrate rigidity four different factors have to be recognised. These are:

(1) Gowers' and Flechsig's spino-cerebellar tracts carrying proprioceptive impulses to those parts of the cerebellum (palæo-cerebellum) which when faradised produce increased muscle tone (Horsley, MacNalty, Bremer).

(2) A secondary vestibular bundle leaving the nucleus fastigii and passing probably partly through and below the lateral wing of the posterior longitudinal bundle to reach the nucleus anterior of the thalamus. The exact identity of this bundle and its postulated crossing in the velum is as yet a matter of debate. Probst and Luna described this tract as an "accessorische Bindarm," but van Gehuchten, Wallenberg, Allen and others are not convinced of its existence. In birds, however, Frenkel(15) has put the existence of the tractus cerebello-diencephalicus as an uncrossed part of the brachium conjunctivum beyond doubt. Physiologically speaking some such connection must be postulated, for I have convinced myself that in several cats after cross-section of the brachium conjunctivum, particularly if the lesion be on both sides, forced movements in the vertical plane (staggering, falling backwards, opisthotonos, increased tone in extensors or occasionally falling forwards) ensued, just as after a lesion of the fastigial nucleus.

(3) The principal cerebellar bundle, *i.e.* tractus dentato-rubralis.

(4) That part of the brachium conjunctivum that is built up by the descending fibres which originate in the basal longitudinal bundle (Wallenberg).

The case to which I would draw attention is a cat in which on May 3rd, 1914, a lesion was affected on the lamina medullaris lateralis thalami on the right side. A degeneration

in Forel's field followed, probably lesion of the tractus pallido-commissuralis. This lesion caused very little alteration in the behaviour of the animal except for some deviation in the horizontal plane.

In this operation the internal capsule escaped any injury; this was proved by the absence of degeneration in the pes pedunculi. On May 5th formol was injected in the endeavour to cause degeneration of the nuclei tecti. This attempt succeeded so far, both nuclei being affected, for the injection entered in the mid-line between the two nuclei and degeneration was found in this commissure and both nuclei tecti. After this lesion the animal demonstrated backward movement, retrograde culbutation or creeping forward crouched low on the floor. In this operation probably secondary vestibular (fastigial) fibres on both sides were interrupted.

It was next (May 18) attempted to cut both basal longitudinal bundles just in front of the posterior commissure by a deep mid-ventral incision (Figs. 2, 3 and 4).

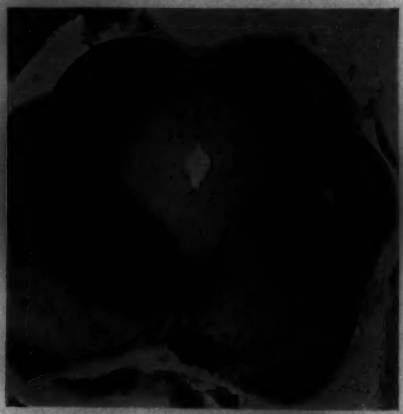


Fig. 2. Section showing most caudal part of mid-brain lesion.

In this case all the vestibular connections associated with vertical movements were severed. In the earlier operations the lamina medullaris externa on the right side and the commissure between both nuclei tecti cerebelli were injured. The condition of decerebrate rigidity which ensued after the last vertical incision (Figs. 2, 3 and 4) (the pyramidal tracts being uninjured, the tr. rubro-spinales showing no degeneration) was more striking than any demonstration of the phenomenon seen by me either in my own or in Horsley's laboratory or elsewhere.

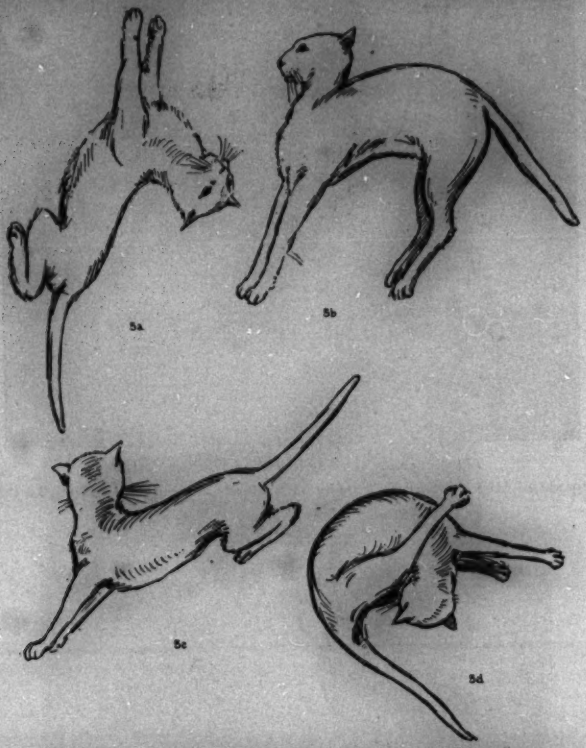
The condition lasted the five days which the animal survived after the last operation, but there were some alterations in posture as indicated in Figs. 5 *a*, *b*, *c*, *d*. If the limbs were moved passively they invariably regained the forced position with a curious spring-like movement.

In this case the red nucleus (Monakow's bundles showed no sign of change) and the pyramidal tracts escaped. It was, however, equally certain that in the final operation the

Fig. 3. Section showing lesion in its greatest extent.
Reproduced from Muskens' *Epilepsy* by permission of Messrs Baillière, Tindall & Cox.

Fig. 4. Section showing otal part of lesion.

interstitial nucleus was injured. This is shown in Fig. 6 where incipient degeneration in the closely adjacent tractus interstitio-spinalis on one side; and on the other side (Fig. 7) in



Figs. 5 *a, b, c, d.* Posture in which cat 242 was found on four subsequent days.

the fibres connecting the commissural nuclei and the globus pallidus is found. (The line of section is directed more anteriorly on the right than on the left side.)

The interpretation of this case of decerebrate rigidity appears to me to be as follows. The rigidity reached the supreme degree because the influence of both nuclei tecti was accentuated in the second operation by the median mesencephalic incision which produced a maximal degree of decerebrate rigidity.

In this case therefore is an example of a double interruption of the secondary vestibular tracts, dealing with forced movements in the vertical plane and frontal plane, including tr. interstitio-spinalis.

Fig. 6. Enlarged. P. L. B. of Fig. 4. Showing incipient degeneration of tr. interstitio-spinalis.

Fig. 7. Enlarged from Fig. 4. Fibres ascending from commissural nuclei towards glob. pallidus on the right side.

With such evidence it must be admitted that the part played by the supra-vestibular connections in the production of decerebrate rigidity is very considerable. This applies especially to the ascending and descending bundles which are associated with movements in the vertical plane.

Sherrington has shown that removal of both labyrinths does not inhibit decerebrate rigidity, and according to Bernis and Spiegel(13) even the destruction of Deiters' nucleus and of the nucleus rami descendens VIII is compatible with the existence of decerebrate rigidity. If, however, the anterior spinal column is interrupted decerebrate rigidity breaks down on the affected side. In such a case the descending tracts from the commissural nuclei (especially the tractus interstitio-spinalis) or Boyce's tract are probably involved.

Summary.

1. In former publications on decerebrate rigidity the writers have dealt with the subject primarily as physiologists, anatomical considerations coming second.

2. Forced movements backwards in the vertical plane, whether caused by a lesion of the primary vestibular centre, by section of the ascending secondary connections, by lesion of the supra-nuclear centre near the posterior commissure, or its palæo-striatal connection are probably always associated with a certain amount of rigidity, recalling Sherrington's "composite postural reflex, the antigravity muscles counteracting the super-incumbent weight."

3. From different observations it is seen that there is a complete reversal of physiological effect at the level of the posterior commissure.

(*a*) In a series of oral to caudal hemisections across the brain-stem of a quadruped temporary rigidity, homo- and hetero-lateral, is noted.

(*b*) If an incision is made in the region of the posterior longitudinal bundle forced movements in the frontal and horizontal plane are directed reversely, accordingly as the incision is on the caudal or oral side of the commissure.

(*c*) The same complete reversal is observed after faradic stimulation of the same region, when oral-caudal sections are made through the brain of primates.

4. Since section of the pyramidal tract has probably nothing to do with the origin of decerebrate rigidity, and as the nucleus ruber is frequently found intact, the part played by the supra-vestibular connections in the production of the phenomenon may be supposed to be considerable. This applies especially to the ascending and descending bundles, lesion of which is associated with forced movements in the vertical plane.

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THE BLOOD-PRESSURE REFLEXES OF THE RABBIT UNDER URETHANE ANÆSTHESIA.

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Urethane is an anæsthetic very frequently used either alone or in combination with other drugs for work on rabbits. The following observations were made in the course of some investigations upon the cerebral circulation, with special reference to the reflex from the carotid sinus extensively described by H. E. Hering(1).

Briefly, according to this author, this reflex is concerned with the regulation of the general blood-pressure level and is subserved by a distinct nerve—the sinus nerve. Stimulation of the sinus region or of the nerve results in a fall of blood-pressure.

Hering, in his monograph, gives many tracings from rabbits, anæsthetised, at least partially, with urethane. It is the purpose of this communication to point out that the effects of this anæsthetic complicate any conclusions drawn from experiments on blood-pressure reflexes in the rabbit.

We are indebted to Sir Charles Sherrington for pointing out to us that depressor reflexes in the rabbit can be obtained from numerous procedures after the administration of large doses of chloral. This fact directed our attention to the urethane.

Intravenous urethane was found to be an excellent anæsthetic for rabbits when recovery, after operation, was desired. For this purpose ·75 grm. per kilo of 25 p.c. solution was slowly injected into an ear vein after which anæsthesia occurred very rapidly. For operative procedures the urethane was supplemented by ether. Recovery was complete from this dose in about 12 hours and without any obvious after effects.

For the acute experiments on reactions obtained from the carotid sinus, the urethane was administered in the same way and in the same or slightly larger dosage[1]. For the operative procedures ether was also

[1] Urethane has been found to have the same action on blood-pressure reflexes when administered intraperitoneally (1·5 grm. per kilo).

given, but was discontinued after the complete setting up of the preparation as it was found that the urethane provided adequate, though light, anæsthesia. It was in this condition that the tracings appended were obtained. When the effects of stimulation were to be compared with those obtained under ether alone, the depth of anæsthesia was regulated so as to be as nearly as possible the same as under the urethane.

Sollman and Brown (2) were the first to describe the fall of systemic blood-pressure consequent on traction on the peripheral end of the common carotid artery. This can be very easily verified in dogs and cats (under ether) and rabbits, and the mechanism has been analysed by Hering. It is present in the rabbit even under ether anæsthesia, but the accompanying tracing (Fig. 1) demonstrates the remarkable effect of the change

Fig. 1. Rabbit. Traction exerted on peripheral end of common carotid artery. Tracing shows slight fall of blood-pressure under ether and the pronounced fall under urethane anæsthesia. Time in seconds.

from ether to urethane anæsthesia. (In all cases the blood-pressure was measured in the femoral artery.)

With ether, traction on the peripheral end of the left common carotid artery produces a slight but perceptible fall. With urethane there is a very marked fall. That is to say, the urethane has "sensitised" the reflex, present to a slight degree under ether.

Fig. 2 demonstrates the same mechanism using inflation instead of

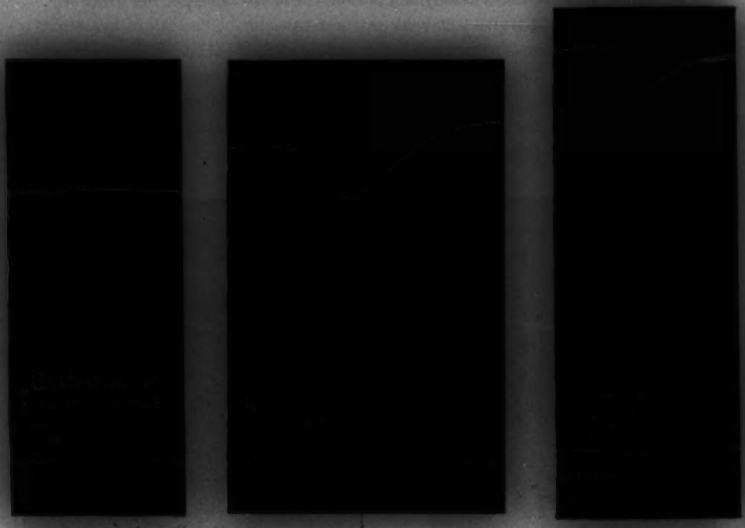


Fig. 2. Fig. 3.

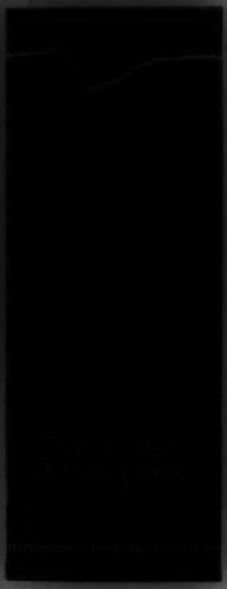
Fig. 2. Rabbit. Shows no effect from distension of the sinus caroticus under ether. A pronounced fall from the same procedure when urethane is substituted.

Fig. 3. Rabbit. Urethane. Traction on the central end of the cut brachial plexus. Shows depressor effect and slowing of the heart. Time in seconds.

traction as a mode of stimulus. In this experiment the internal carotid was tied as near the skull as possible. The external carotid was tied well above the bifurcation and all the small arterial branches visible in the neighbourhood were ligated. The sinus region was then inflated with saline through a syringe connected to a cannula inserted headwards into the common carotid artery. Under ether anæsthesia there was no perceptible reaction during inflation. When the change had been made to urethane a pronounced fall accompanied the distension. It was also

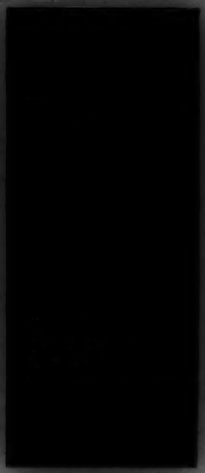
found that traction on the central end of the cut brachial plexus would produce, under urethane, a well-marked fall of pressure. This fall could be elicited after the removal of the stellate ganglion and the cutting of both vagi in the neck, or the administration of atropine. However, with the vagi intact there was also a slowing of the heart (Fig. 3). Under ether, traction on the plexus produced either a slight rise of pressure or no effect.

An interesting point associated with this depressor effect is the spontaneous recovery of the blood-pressure level which takes place despite the continuance of the stimulus (stretch or faradisation, Fig. 4).



a

Fig. 4.



b

Fig. 5.

Fig. 4. *a*. Rabbit. Urethane. Shows return to normal blood-pressure during continuance of traction stimulation. *b*. Rabbit. Urethane. The same effect with faradic stimulation. Time in seconds.

Fig. 5. Rabbit. Urethane. Depressor effect from traction on the central end of the subclavian artery. Atropine given 7 minutes beforehand.

Traction on the central end of the subclavian artery was also found to produce a fall of pressure under urethane (Fig. 5) but this was analysed as being due to the traction on the artery being transmitted to the adjacent brachial plexus. Traction on a muscle in the rabbit (opening the jaws or stretching the quadriceps femoris) gives no effect or a rise in blood-pressure under ether anæsthesia. When however urethane has been substituted a depressor effect is obtained (Fig. 6).

Fig. 6. Rabbit. Under ether traction on the quadriceps femoris produced, in this case, a rise of pressure. When urethane was substituted for ether there was a fall of pressure from the same procedure. Time in seconds.

Fig. 7 shows the result of faradisation of the central end of the femoral nerve with urethane anæsthesia. There is a considerable fall of blood-pressure.

It is clear, from the tracings and explanation given, that urethane, even when only producing light anæsthesia, is capable of influencing the rabbit's reactions so that depressor effects are obtained from a

variety of stimuli. The experiments herein described offer no explanation as to the mode of action of the urethane but, in view of the widespread

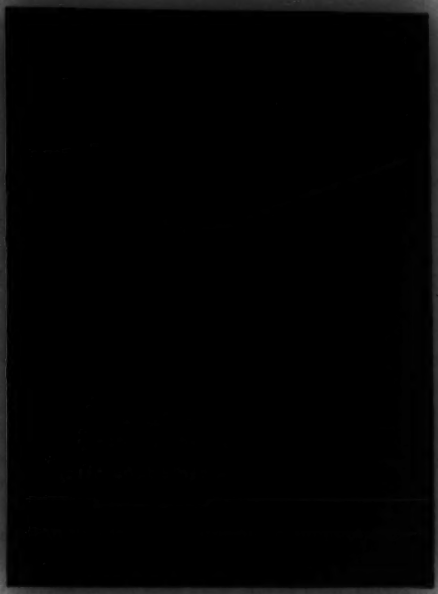


Fig. 7. Rabbit. Urethane. Shows the depressor effect from stimulation of the central end of the femoral nerve.

use of this drug, it was thought desirable to call attention to this disturbing factor in the evaluation of results.

Summary.

Under urethane anæsthesia a variety of stimuli, in the rabbit, produce depressor reflexes which are slight or absent under ether anæsthesia.

This work was done by H. F. while working under the Freedom Research Fund of the London Hospital.

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THE ORIGIN OF THE GLUCOSE IN THE HYPERGLYCÆMIA INDUCED BY PITUITRIN.

By G. A. CLARK.

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The injection of extracts of the posterior lobe of the pituitary either intravenously or subcutaneously into a normal animal produces usually an increase in blood-sugar which may last from one to two hours or, in some cases, longer, depending on dosage, and the type and condition of animal; occasionally the hyperglycæmia is negligible and in a few instances the blood-sugar has been found to fall (1, 2). While it is accepted that the usual action of pituitrin[1] is to produce hyperglycæmia, there is no satisfactory evidence to show how the effect is brought about. Moehlig and Ainslie (3), however, have suggested that the source of the additional sugar is the muscle glycogen, which they believe may be liberated in an indirect manner by the action of pituitrin on the suprarenals. If pituitrin can set free glycogen from the muscles in this way, it appeared that the decerebrated, eviscerated animal was a suitable preparation in which to investigate the problem.

The preparations were made from cats according to the method described by Burn and Dale (4) and the continuous glucose infusion apparatus of these authors was used to maintain as nearly as possible a constant blood-sugar level prior to the injection of pituitrin; glucose infusion was continued throughout the experiment at the same rate. The concentration of the solution was 1·5 p.c. and the rate of infusion required varied in different animals from 1 c.c. in 2·9 mins. to 1 c.c. in 4 mins. The temperature of the preparation was maintained by means of an electrically heated table, hot water bottles and suitable felt wrappings which formed a tent over the animal and contained a warming bulb. Blood for sugar estimations was withdrawn from one femoral vein and pituitrin injections were made into the femoral vein of the opposite limb. The dose of pituitrin was in every case 1 c.c.

[1] The pituitrin used and referred to throughout the paper is the preparation issued under that name by Parke, Davis and Co.

From Table I it is seen that in no experiment was the sugar level raised by pituitrin but, on the contrary, a very definite fall was produced. This fall was not evident till the second half-hour in some cases while in others it was apparent during the first half-hour after pituitrin. Control experiments in which no pituitrin was given showed no such abrupt changes in sugar level over the same period of time (Exps. 7 and 8).

TABLE I.

		Before pituitrin			After pituitrin		
Exp.	Hours ...	1	½	0	½	1	1½
1		·330	·320	·310	·266	·231	—
2		·242	·238	·230	·219	·206	·194
3		·294	·291	·288	·288	·259	—
4		·250	·253	·254	·250	·206	·197
5		·204	·210	·218	·223	·210	·200
6		·418	·419	·426	·412	·346	·325
7 (control)		·326	·321	·314	·311	·305	·296
8 (control)		·262	·265	·265	·267	·264	·270

In every case except Exp. 5 the suprarenals and their blood-supply were left intact; in Exp. 5 the glands were excluded from the circulation by a pedicle ligature, without in any way altering the subsequent response to pituitrin. The sudden increase in the rate of fall of glucose concentration in this series of experiments may be explained by (*a*) dilution of the blood, (*b*) increased oxidation of glucose, or (*c*) building up or decreased breaking down of muscle glycogen.

In support of the first of these possible explanations there are the observations of Craig(5) on human beings and of Underhill and Pack(6) on dogs that the administration of pituitary extract in conjunction with the exhibition of large quantities of water does cause a diminution in hæmoglobin content of the blood, while Partos and Katz-Klein(7) obtained a definite dilution of the blood in rabbits as a result of the injection of pituitary extract and showed that this dilution may mask the hyperglycæmia usually seen in the intact animal. In the present series of experiments the hæmoglobin content of the blood was determined at intervals before and after pituitrin in three preparations and in two of them blood-sugar estimations were made at the same time. The results are shown in Table II.

It is obvious that in Exp. 4 blood dilution cannot account for the observed fall in blood-sugar and that in Exp. 9 the 5 p.c. dilution following the injection of pituitrin would be insufficient to account for the fall of sugar concentration in any of the experiments in Table I. In Exp. 6 where the concentrations of both sugar and hæmoglobin are seen to fall after the dose of pituitrin it is necessary to examine the changes

TABLE II.

		Before pituitrin			After pituitrin		
Exp.	Hours ...	1	½	0	½	1	1½
4	Hæmogl. p.c.	68	70	69	68	67	64
	Glucose	·250	·253	·254	·250	·206	·197
6	Hæmogl. p.c.	88	87	85	82	75	73
	Glucose	·418	·419	·426	·412	·346	·325
9	Hæmogl. p.c.	—	60	57	56	54	54
	Glucose	—	—	—	—	—	—

more closely. If curves are drawn to show the percentage change in the initial concentrations throughout the experiment (Fig. 1) it is seen that before pituitrin was injected the sugar concentration was rising slightly and the hæmoglobin falling, both changes clearly the result of the infusion of sugar solution. After pituitrin had been injected the sugar concentration fell more rapidly than that of the hæmoglobin[1]. It is difficult to see how dilution of the blood by tissue fluid could account for this. The fluid in the tissue spaces would contain no hæmoglobin, but at any rate some sugar, an amount of sugar, in fact, determined by the amount in the blood. Unless, therefore, the capillaries can take up

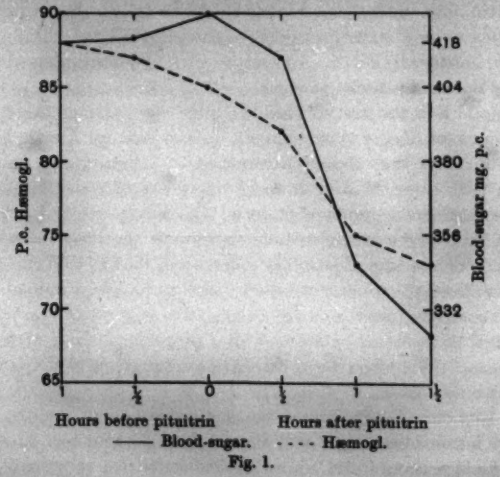

Fig. 1.

fluid from the tissue spaces in such a way as to exclude the sugar which that fluid contains, a supposition for which no evidence exists and which

[1] I am indebted to Mr A. Spencer for carrying out hæmoglobin estimations.

it is very difficult to allow, the dilution of the blood by tissue fluid must lower the concentration of hæmoglobin more than that of the sugar. The figures in this experiment and the curves in Fig. 1 show that, on the contrary, the concentration of sugar after pituitrin even in this experiment is lowered more than that of the hæmoglobin. The fall of blood-sugar therefore cannot be accounted for by blood dilution. Whatever be the cause of the disappearance of glucose from the blood in eviscerated animals following the injection of pituitrin, it is obvious that the muscles are not the source of the additional sugar in pituitrin hyperglycæmia. Whether pituitrin has any specific action on carbohydrate metabolism in muscle is not known, but the experiments of Ahlgren(8) suggest the possibility. Using Thunberg's methylene blue method for measuring tissue oxidation, he showed that some samples of hypophyseal extract definitely increased the oxidation processes in muscle; with other samples he found a decrease.

The results so far described are of interest in view of the findings of Tingle and Imrie(9) that pituitrin temporarily lowers the blood-sugar in diabetics. Moreover, in two of the cases reported by these authors there is a further similarity in that the fall was not evident till the second half-hour.

Further confirmation of the view that pituitrin cannot liberate glucose from the muscles was obtained in three experiments on the decerebrated and eviscerated cat in an attempt to demonstrate the antagonism between pituitrin and insulin. 1 c.c. of pituitrin was injected intravenously half an hour after the administration of 7 clinical units of insulin. It is evident from Table III that in no case is the rate of fall of blood-sugar retarded by the pituitrin.

TABLE III.

		Before insulin			After insulin		
Exp.	Hours ...	1	½	0	½	1	1½
10		·213	·204	·196	·174	·133	·109
11		·275	·284	·292	·254	·222	·204
12		·305	·321	·341	·274	·248	—

The abrupt rise in sugar level following the injection of pituitrin in the normal animal suggests a sudden glycogenolysis rather than a temporary suppression of the normal rate of utilisation of glucose. It was natural therefore to consider the liver as the possible seat of action. For this investigation cats under amytal anæsthesia were used, the liver was excluded from the circulation by anastomosing the portal vein with the

central end of one renal vein, in one experiment by means of a suitably shaped paraffined glass cannula and in two others by glass cannulæ connected by tubing after injecting hirudin intravenously[1]. All other structures in the hepatic pedicle were cut between ligatures. In all three cases the animals lived for two hours after operation. The blood-sugar of the cat without hirudin was high and fell rapidly, that of the two where hirudin had been given was low and fell less rapidly. The rate of fall was determined in the latter by two, and in the former by three estimations at intervals of half an hour. 1 c.c. pituitrin was then injected intravenously and sugar estimations continued. In no case was any great change in the rate of fall of blood-sugar produced (Table IV), although in Exp. 13 the rate appeared to be accelerated.

TABLE IV.

		Before pituitrin			After pituitrin	
Exp.	Hours ...	1	½	0	½	1
13		·245	·222	·181	·127	·103
14		—	·108	·097	·078	·063
15		—	·104	·080	·065	·041

Although the short duration of these experiments was unsatisfactory it appeared that the production of pituitrin hyperglycæmia was not possible in the absence of the liver, and the two following experiments suggest that the degree of hyperglycæmia is governed by the amount of available glycogen in this organ. The normal response of a rabbit on a full diet to 1 c.c. pituitrin was determined (Exp. A) and then the animal's diet was reduced and repeated injections of strychnine given over a period of three days, when the reaction of the blood-sugar to the same dose of pituitrin was investigated (Exp. B). In the second case a less degree of hyperglycæmia was found.

Exp.	Hours ...	0	½	1	1½	2	2½	3
A		·101	·161	·141	·113	·109	·103	—
B		·109	·135	·103	·117	·113	·107	·106

The question of how pituitrin liberates liver glycogen has not so far been capable of definite answer, but it is apparent that the sympathetic nervous system plays no important part in the action because, in several experiments in rabbits which had previously received a dose of ergotamine tartrate sufficient to prevent adrenaline hyperglycæmia, pituitrin still produced an increase in blood-sugar comparable in degree to that

[1] I am indebted to Dr Trevan for a supply of hirudin.

produced in the absence of ergotamine (10). Moreover, in Table V are given the hyperglycæmic responses to pituitrin in cats, under amytal anæsthesia, after operative procedures which eliminate sympathetic action on the liver. In Exp. 17 both suprarenal glands were removed by incising the peritoneum, raising the glands and tying a ligature round the pedicle prior to injecting pituitrin. The same operation was done in Exp. 18, but in addition all nervous connections of the liver in the hepatic pedicle were cut. In both cases pituitrin produced hyperglycæmia greater, rather than less, than that observed in a control animal (Exp. 16). Exp. 19 shows the response to pituitrin in a cat under amytal in which the hepatic artery was tied, and here again the effect is not diminished.

TABLE V.

Exp.	Hours ...	0	½	1	1½	2	2½	3
16		·100	·176	·135	·098	·090	·091	—
17		·125	·165	·176	·122	·084	·074	·083
18		·113	·182	·140	·107	·103	·093	—
19		·118	·202	·159	·142	·111	·092	—

It thus appears that, although pituitrin acts on the liver glycogen reserves, its action does not depend on the sympathetic nervous system either directly or indirectly by way of the suprarenals; nor is the blood-supply from the hepatic artery an important factor. It may be, then, that pituitrin exerts its effect either (1) directly on the liver cells, (2) indirectly by the production of some substance which is carried to the liver in the portal blood, or (3) by so altering the blood-supply to the liver that glycogenolysis is temporarily increased. An investigation of these alternatives will form the subject of a further paper.

SUMMARY.

1. The intravenous injection of pituitrin into decapitated, eviscerated cats increases the rate of disappearance of sugar from the blood. The antagonism between insulin and pituitrin is not evident.

2. In cats under amytal anæsthesia, in which the liver is cut out of the circulation, pituitrin does not produce the hyperglycæmia seen in normal animals, nor does it diminish the rate of fall of blood-sugar.

3. From these observations it appears that the source of glucose in pituitrin hyperglycæmia is the liver glycogen.

4. Experiments are described which appear to exclude (*a*) the sympathetic nerve-supply to the liver, (*b*) the suprarenal glands, (*c*) the blood-supply to the liver by way of the hepatic artery, as intermediary factors in the production of pituitrin hyperglycæmia.

I wish to thank Professor Leathes for his unfailing interest and helpful criticism in this work.

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BILE SALTS AND SECRETIN AS CHOLAGOGUES.

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The hypothesis of the circulation of the bile is founded mainly on observations made by Schiff(1) in 1868. Schiff established, in dogs, a large fistulous opening between the surface of the abdomen and the gall bladder without the common bile duct being ligatured. Under these circumstances the bile usually flows externally as long as the exit is free. If, however, the cannula fitted into the fistula be closed, then the bile flows into the intestine. Such fistulæ were called "amphibolic" by Schiff. He found that the quantity of bile obtained in a given period from the fistula was always much greater immediately after the bile had been allowed to flow into the intestine than when it had been allowed to flow externally. Further, he observed that the introduction of bile salts into the intestine through a duodenal fistula increased the total quantity of bile secreted. Upon these facts Schiff based his hypothesis of the circulation of the bile—that a portion of the bile absorbed from the intestine, on reaching the liver in the portal blood, supplies the material for a fresh secretion of bile.

In 1902 Bayliss and Starling(2) showed that the intravenous injection of secretin freed from bile salts caused not only a copious secretion of pancreatic juice, but also doubled the rate of flow of bile from the liver. In the experiment quoted the spontaneous secretion of bile was 27 drops in 15 min.; this was increased to 42 drops in 12 min. after the injection of the secretin solution.

In 1924 I(3) showed that the introduction of bile of an adequate reaction into the duodenum of a cat caused a large secretion of pancreatic juice, extending in some cases over a period of three hours. An analysis of this phenomenon showed that the immediate stimulus to the pancreas was secretin which had been carried by the absorbed bile salt into the portal blood. These observations indicated that the increased flow of bile observed by Schiff after the introduction of bile into the duodenum might have been due to the action of secretin on the liver

cells in a manner comparable to the action of secretin on the cells of the pancreas. This hypothesis would correlate the observations of Schiff with those of Bayliss and Starling and would explain why substances so dissimilar as bile salts and secretin stimulate the liver cells to secrete bile.

Experimental Methods. The majority of the results recorded were obtained from experiments on cats. In certain instances rats, which possess no gall bladder, were used as the experimental animals. All the animals were anæsthetised by a hypodermic injection of veronal (0·4 grm. per kilo of body weight). During the preliminary operations, chloroform was given, especially in the case of old cats in which the anæsthetic effect of veronal is delayed. Cannulæ were tied into the gall bladder, the common bile duct, and the pancreatic duct. Intravenous injections were made by means of a cannula tied into the right femoral vein and arterial blood-pressure records were obtained from the femoral or carotid artery. The rates of secretion of bile and pancreatic juice were timed by a stop watch.

The natural flow of freshly secreted bile in the cat is from the liver down the common bile duct. If, however, the path down the common bile duct is obstructed, either through the contraction of the duodenal muscle round that portion of the duct which passes through the intestinal wall or through some other cause, then the bile is diverted along the cystic duct to the gall bladder. When a cannula is placed in the common bile duct there is no obstruction to the flow and the freshly secreted dilute bile may be collected from it. This fact is of importance since a fundamental difficulty in determining the factors responsible for the secretion of bile is the presence of a gall bladder, capable of considerable variations in capacity, on the path of the secretion. The constancy of the level of bile in the cannula tied into the gall bladder showed that all the bile secreted by the liver passed directly down the common bile duct. Results obtained from animals in which the secreted bile came from the gall bladder owing to some obstruction in the common bile duct were rejected. The spontaneous variations in the capacity of the gall bladder are so considerable that such observations on biliary secretion over definite intervals of time are unreliable.

Bile as a Cholagogue.

(1) *The injection of bile into the duodenum.* The following figures give the amounts of bile and pancreatic juice secreted after the injection of dilute bile (2 c.c. ox bile, 7 c.c. NaCl 0·9 p.c., 1 c.c. $NaHCO_3$ 1·5 p.c.)

into the duodenum of a cat. The cat was in a fasting condition and, as previously described, it was necessary to add bicarbonate to the bile to evoke a secretion of pancreatic juice. There was no spontaneous secretion of pancreatic juice; that of the bile was 10 drops per hr. Thirteen minutes after the injection of the dilute bile, both pancreatic juice and bile were secreted at a rapid rate. The following figures give a synopsis of the results:

Time (hr.)	Pancreatic juice (drops)	Bile (drops)
1st	70	58
2nd	54	62
3rd	9	15
Total juice—5·6 c.c. in 3 hr.		Total bile—5·0 c.c. in 3 hr.

After 3 hr. the flow of pancreatic juice ceased and the bile flow diminished to its spontaneous rate—10 drops per hr. The experiment offers an adequate demonstration of Schiff's statement that the entrance of the bile into the duodenum causes a flow of bile. But it does not justify the conclusion that the injected bile is the immediate cause of the increased bile flow from the liver. In fact the parallelism of the secretions of pancreatic juice and bile appear to suggest that both secretions are due to the same cause—the passage of secretin into the blood.

(2) *The injection of bile into the ileum.* The hypothesis that secretin is the immediate excitant of biliary secretion after the injection of bile into the duodenum was tested by direct experiment. In the carnivorous animal the ileum is practically free from secretin. Therefore, if the absorption of bile injected into this part of the intestine is followed by an increased secretion of bile from the liver, it is evident that secretin cannot be regarded as the sole agent in the secretion of bile. Slightly acidified bile (2 c.c. ox bile, 8 c.c. NaCl 0·9 p.c., 0·05 c.c. HCl(N)) was injected into the ileum of a fed cat. After 26 min. bile was rapidly secreted at the following rate:

Bile (drops)	Min.	sec.
6	6	50
12	15	10
24	32	35
36	51	2
48	70	0
60	96	0

The increased secretion of bile continued for 2·5 hr. during which time 3 c.c. of bile were secreted. There was no secretion of pancreatic juice during the whole of this time. It is evident therefore that the liver

secretes bile after the injection of bile into the small intestine quite independently of the absorption of secretin into the blood. The result does not exclude secretin as a stimulus to the biliary activity of the liver, but it proves that the secretion of bile may be augmented independently of the passage of secretin into the blood.

(3) *The intravenous injection of extracts of ileum.* The previous results indicate that two secretins may exist, one for the pancreas, and the other for the liver, and that whereas the former is limited to the duodenum, the latter may extend along the whole length of the small intestine. On this hypothesis bile salts absorbed from the duodenum carry both secretins into the blood and cause the secretion of both pancreatic juice and bile, whilst bile salts absorbed from the ileum carry the hepatic secretin only into the blood and hence cause the secretion of bile only. This hypothesis was tested by making extracts of the mucosa of the ileum with various solvents (water, alcohol, dilute acid, dilute alkali, 0·9 p.c. NaCl, etc.) and observing the effects of the intravenous injection of these extracts on biliary secretion. In no case was there any increase in the bile secreted. Exhaustive experiments gave no evidence in support of the hepatic secretin hypothesis.

(4) *The intravenous injection of bile.* The following experiment showed that the action of bile in the intestine as a cholagogue is directly due to the absorption of bile into the portal blood. 1 c.c. of ox bile diluted with 5 c.c. of 0·9 p.c. NaCl was injected into the femoral vein of a cat. The spontaneous secretion of bile was at the rate of 16 drops per hr.

Time (min.)	A. Bile secreted (drops)	B. Spontaneous secretion (drops)
15	8	4
30	23	8
45	35	12
60	46	16
90	53	24

Column B is inserted so that the amount of bile secreted after the intravenous injection of bile (column A) may be compared with the quantity which would have been secreted spontaneously owing to the continuous activity of the liver. The figures show that the intravenous injection of bile (1 c.c.) increased the secretion of bile for 1 hr., a threefold increase being recorded in the first 45 min.

(5) *The intravenous injection of sodium cholate.* An analysis of the above experiment shows that cholalic acid is the substance present in bile which stimulates the liver to secrete more bile. The effect on biliary

secretion of the intravenous injection of 0·1 grm. of cholalic acid, dissolved in dilute NaOH, is given in the following figures:

Time (min.)	Bile secreted (drops)	Spontaneous secretion (drops)
15	10	2
30	24	4
45	41	6
60	48	8

The enhanced secretion of bile terminated after 1 hr., as in the previous experiment. In these last two experiments the spontaneous secretion of bile was small since the cats were in a fasting condition.

The results of the experiments described in this section show that the absorption of bile salts (cholalic acid) from the intestine into the blood, apart from the synchronous absorption of pancreatic secretin (or hypothetical hepatic secretin), increases the amount of bile secreted by the liver. The experiments fully confirm Schiff's original hypothesis of the circulation of the bile.

Secretin as a Cholagogue.

Bayliss and Starling showed that the intravenous injection into a dog of secretin contained in a 0·2 p.c. HCl extract of the duodenal mucous membrane doubled the secretion of bile by the liver. Bile salts were removed from the duodenal mucous membrane by alcohol before making the secretin extracts. My experiments indicate that secretin is contained in a preformed condition in the duodenal mucous membrane and is at least as soluble in alcohol as bile salts. Therefore, in view of the marked cholagogue action of 0·1 grm. of cholalic acid (cf. *supra*), it appeared advisable to determine the capacity of purified secretin to cause a secretion of bile.

(1) *Properties of purified secretin.* I(4) have previously given a brief description of the method by which purified secretin may be obtained. The preparation possesses a high degree of activity when tested on pancreatic secretion. It contains no trace of bile salts and has no action on the general blood-pressure. This latter fact is of importance since changes of blood-pressure that alter the amount of blood in the liver might directly bring about the retention or expression of bile from the bile canaliculi and ducts.

(2) *The intravenous injection of secretin.* This preparation of secretin, when injected into the blood stream in sufficient quantity to produce a large flow of pancreatic juice, augments to a small degree the secretion

of bile by the liver. The small increase in the bile flow is invariably produced whether the spontaneous secretion of bile is large or small. The following figures show the effect on the secretions of pancreatic juice and bile of an injection of 0·1 mgrm. of secretin (dissolved in 1 c.c. H_2O) into the femoral vein.

Time (min.)	Pancreatic juice (drops)	Bile (A) (drops)	Bile (B) spontaneous secretion (drops)
5	12	4	3
10	26	9	6
15	37	13	9
20	41	16	12
30	—	21	18

Column B shows the spontaneous secretion of bile on the assumption that the factors which cause this constant secretion persist after the intravenous injection of secretin. The figures show that pancreatic juice was rapidly secreted for 20 min. (41 drops), and during that time the secretion of bile was augmented by 4 drops. The increased secretion of bile may be much less than that observed by Bayliss and Starling.

(3) *The destruction of secretin.* The question arose whether it was one and the same principle in the secretin preparation that excited the secretions of both pancreatic juice and bile, or whether two different secretins, pancreatic and hepatic, were contained in it. It has been shown previously that pancreatic secretin is destroyed by pepsin, trypsin, or hydrolysis by weak acid or alkali. The partial or complete destruction of the pancreatic action of the secretin preparation by any of the above means results in a corresponding change in the cholagogue action. The results indicated that the same active principle causes the flow of pancreatic juice and the increase in the spontaneous secretion of bile.

(4) *Repeated injections of secretin.* Many observations suggested that the cholagogue action of secretin was apparent rather than real—that secretin caused a little bile to be squeezed out of the liver either by increasing the size of the liver cells or by altering to some small degree the amount of fluid (blood or lymph) contained in the liver. The average weight of a cat's liver is 100 grm., whilst the increased secretion of bile, produced by a quantity of secretin which causes a maximal secretion of pancreatic juice, amounts to about 0·5 c.c. Therefore only small changes in the condition of the cells or fluid content of the liver would expel this extra quantity of bile from the canaliculi or ducts. The type of experiment which supported this conclusion is given:

0·5 mgrm. of secretin was injected every 45 min. into the femoral

vein of a fasting cat. The spontaneous secretion of bile was at the rate of 4 drops of bile per hr. The quantities of pancreatic juice and bile, and the latent periods between the injection of secretin and the start of the secretion of pancreatic juice and the increased secretion of bile, are given.

	Secretin (mgrm.)	Pancreatic juice	Latent period (secs.)	Bile	Latent period (min.)
1st injection	0·5	46 drops in 30 min.	50	13 drops in 32 min.	10
2nd injection	0·5	52 drops in 36 min.	45	13 drops in 45 min.	10
3rd injection	0·5	54 drops in 38 min.	45	14 drops in 42 min.	5

The cessation of bile secretion after varying times (32 min., 45 min. and 42 min.), and the long latent period before a fresh injection of secretin initiated a renewed flow of bile, offered some support to the hypothesis of the mechanical expulsion of bile from the canaliculi in the liver.

In order to obtain other evidence bearing upon this hypothesis an experiment was done in which injections of secretin (0·1 mgrm.) were repeated every 30 min., that is, at intervals of time during which the apparent cholagogue action of secretin manifests itself. On the mechanical hypothesis of the action of secretin successive injections, whilst producing the same quantity of pancreatic juice, should produce diminishing quantities of bile.

Time of injection (p.m.)	Pancreatic juice (drops)	Bile (drops)
2.30	16	21
3.0	17	20
3.30	18	20
4.0	20	21
4.30	20	21

The spontaneous secretion of bile was at the rate of 10 drops in 31 min. The constancy of the augmented secretion of bile (10 drops every 30 min.) proved that this augmented secretion was not secondary to some mechanical action, either vascular or cellular, by which previously secreted bile contained in the bile canaliculi or ducts was squeezed out of the liver.

(5) *Removal of small intestine.* The long latent period which elapses between the injection of secretin and the augmentation of biliary secretion seemed to indicate that the cholagogue action was secondary to an effect on some other tissue. An apparent increase in intestinal movements after the intravenous injection of secretin suggested that accelerated absorption into the blood of bile salts contained in the small intestine

was the immediate cause of the increased secretion of bile. The assumption was tested by an experiment in which the cholagogue action of secretin, before and after removal of the small intestine, was determined. The intravenous injection of 1 mgrm. of secretin into a cat caused the secretion of 46 drops of pancreatic juice and 22 drops of bile in 40 min. The small intestine was removed and the same quantity of secretin injected. In the succeeding 40 min. 34 drops of pancreatic juice and 14 drops of bile were secreted. The amount of bile secreted after partial evisceration was diminished, but the diminution was of the same order as that of the pancreatic juice and was probably due to the shock caused by the removal of the small intestine which immediately preceded the second injection. There was no evidence that the cholagogue action of secretin was the result of an increased absorption of bile salts from the intestine.

(6) *Effect of clamping the pancreatic vein.* As a corollary to the previous experiment, the effect of preventing or diminishing the blood flow through the pancreas, before and after the injection of secretin, was determined. Two experiments are recorded in which (*a*) the pancreatic vein was ligatured and no collateral circulation was established in the pancreas, and (*b*) the pancreatic vein was clamped, but a collateral circulation was established after 15 min. as evidenced by a small secretion of pancreatic juice.

(*a*) In the following experiment the spontaneous secretion of bile, and the secretions of bile and pancreatic juice caused by the intravenous injection of 0·1 mgrm. of secretin before and after the ligation of the main pancreatic vein are recorded:

	Pancreatic juice (drops)	Bile (drops)
Normal flow for 30 min.	0	10
Secretion during 30 min. after 0·1 mgrm. of secretin	20	20
Pancreatic vein ligatured:		
Secretion during 30 min. after 0·1 mgrm. of secretin	0	11

The occlusion of the main pancreatic vein does not prevent the access of secretin to the liver cells by the hepatic artery. Since, under these conditions, secretin not only produces no pancreatic juice, but does not augment the spontaneous secretion of bile, it may be assumed that the prime action of secretin is on the cells of the pancreas, and that the cholagogue action is due to some product of pancreatic activity which passes via the blood to the liver and excites it to secrete bile.

(*b*) This conclusion was confirmed by an experiment in which a collateral circulation was established after clamping the main vein.

	Pancreatic juice (drops)	Bile (drops)
Normal flow for 1 hr.	0	44
Flow in 1 hr. after 0·2 mgrm. secretin	53	67
Pancreatic vein ligatured:		
Flow in 1 hr. after 0·2 mgrm. secretin	17	52

A collateral circulation through the pancreas was established 15 min. after the injection of the second quantity of secretin since, after this period, a small secretion of pancreatic juice started. The comparative figures bring out an interesting fact—the first secretin injection produced 53 drops of pancreatic juice and augmented the biliary secretion by 23 drops; the second secretin injection, after clamping the pancreatic vein, produced 17 drops of pancreatic juice and augmented the bile secretion by 8 drops. The quantities of pancreatic juice and bile bear about the same ratio to one another in both cases: *i.e.* 53 to 17 and 23 to 8. The results indicate that the action of secretin as a cholagogue is a secondary effect depending upon its action on the pancreas. Secretin acts on the pancreas causing a secretion of pancreatic juice; as a result of this glandular activity metabolic products pass into the portal blood and are carried directly to the liver; these metabolic products from the pancreas excite the biliary activity of the liver and cause an increased secretion of bile.

Summary.

(1) The injection of bile into the duodenum causes a large secretion of pancreatic juice and augments the secretion of bile.

(2) A similar injection of bile into the ileum augments the secretion of bile but has no effect on pancreatic secretion.

(3) The intravenous injection of bile salts augments the flow of bile but has no effect on pancreatic secretion.

(4) The intravenous injection of purified secretin causes a large secretion of pancreatic juice and augments the flow of bile.

(5) The action of secretin as a cholagogue is annulled by procedures which prevent the secretion of pancreatic juice.

(6) These facts show that (*a*) bile salts, free from secretin, absorbed into the blood from the small intestine act as cholagogues; (*b*) the action of secretin as a cholagogue is secondary to its action on the pancreas.

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THE SIGNIFICANCE OF THE DIASTOLIC AND SYSTOLIC BLOOD-PRESSURES FOR THE MAINTENANCE OF THE CORONARY CIRCULATION.

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It is generally agreed that the arterial blood-pressure is the main circulatory factor determining the coronary blood flow, but as regards the relative importance of the diastolic and systolic pressures opinions differ. Starling and his co-workers (1, 2, 3) find that the coronary circulation is within wide limits independent of changes in pulse pressure provided that the *mean* aortic pressure is kept constant. On the other hand, Smith, Miller and Graber (4) state that the maintenance of an efficient coronary circulation is fundamentally dependent on the height of the *diastolic* pressure, and that only in experiments in which the diastolic pressure is reduced to a permanent low level can the coronary blood flow be affected by changes in the systolic pressure. Starling's measurements of blood-pressure were in most cases made with the mercury manometer. Smith, Miller and Graber used a membrane manometer the vibration frequency of which was not stated, but, as can be judged from the tracings given in their paper, it was probably not high.

The determination of the relative significance of the systolic and diastolic blood-pressures is of considerable importance for a correct comprehension of the blood supply to the heart muscle. It is especially important in conditions in which the pulse pressure changes without a material alteration in the mean pressure as may occur, for instance, as a result of acceleration of the heart beat or of a reduction in the arterial resistance with a simultaneous increase in the systemic output or in the case of aortic regurgitation.

The experiments described below were performed with the object of securing further information on this point. They were performed on the heart-lung preparation, since in this preparation the diastolic and systolic pressures can be easily and independently controlled and maintained constant for any desired length of time.

The blood-pressure was measured immediately above the aortic valves with an optical manometer, the membrane of which had in the different experiments a vibration frequency of 150–170 and a sensitivity such as to give, at the magnification used, a deflection of 1 mm. for each mm. Hg change in pressure. The blood flowing from the drained coronary sinus was collected and measured during intervals of 1 min. and was considered constant if five successive measurements differed by not more than $\pm$ 0·5 c.c. The diastolic and systolic pressures were changed by varying (*a*) the systemic output or (*b*) the heart rate which was in all experiments controlled by rhythmic stimulation of the right auricle. The actual experiments proceeded as follows. Optical records of the aortic pressure were obtained after carefully measuring the coronary blood flow and the systemic output of the heart. The output was then increased while the artificial arterial resistance was adjusted so as to maintain (say) the systolic pressure at the level it had during the preceding period of small output. The aortic pressure and the coronary blood flow were again recorded. The observation was then repeated, keeping in this case the diastolic pressure constant. Now, still maintaining a large output, the arterial resistance was adjusted to a level at which the coronary blood flow was within $\pm$ 0·5 c.c. the same as it had been during the period of the small output, a further set of optical records of the aortic pressure being taken. We thus had four sets of records of the aortic pressures and of the coronary blood flow in each experiment; the first (1) which was taken during the period of small output and the rest (2, 3 and 4) during the period of larger output, (2) with the same systolic pressure, (3) with the same diastolic pressure and (4) with an aortic pressure such that the coronary blood flow during the period of large output was equal to that during the period of small output. In order to determine whether the condition of the coronary blood vessels had undergone a change during the 15 to 20 min. of observation, the output and pressure were again returned to their original values, only those experiments being used in which the coronary blood flow during this control period was equal to that observed before the increase in the output. The above four sets of observations could usually be repeated several times on the same heart-lung preparation. Similar observations were made in experiments in which the pulse pressure was changed by varying the heart rate while the systemic output was maintained constant.

The Effect of Changes in Output.

The results of our experiments with changes in output were concordant in showing that neither the diastolic nor the systolic pressure alone determines the coronary circulation. Thus our observations fail to support the conclusions reached by Smith, Miller and Graber; we find that the mean aortic pressure is in every case a far more significant factor in the coronary circulation. Table I gives the results of a typical experiment.

Table I.

Dog—10 kl.; heart—62 grm. The heart rate was maintained throughout this experiment at 122 beats per min. and the temperature at 37° C.

Systemic output in c.c. per min.	Diastolic pressure in mm. Hg	Systolic pressure in mm. Hg	Arithmetical mean pressure in mm. Hg	Blood flow from the coronary sinus in c.c. per min.
(a) The diastolic pressure is maintained constant.				
1170	80	179	129½	142
890	80	158	119	99
465	80	130	105	68
200	80	118	99	57
(b) The systolic pressure is maintained constant.				
200	80	118	99	56
460	65	118	91½	42
885	42	118	80	31
1160	20	118	69	18
(c) The coronary blood flow is maintained constant.				
200	80	118	99	57·0
470	70	126	99	56·5
880	60	134	97	56·5
1175	42	147	95	57·0

The first and the second part of the table show that the coronary outflow is affected by changes in the systolic pressure as well as by changes in the diastolic pressure or, in other words, that it is not determined by either of these pressures singly. The coronary blood flow closely follows the changes in the arithmetical mean pressure, a rise in which has a relatively greater effect as the pressure reaches a higher level: a change in the mean pressure from 99 to 129 mm. increased the coronary flow by 85 c.c., while an equal change from 69 to 99 mm. increased it by only 38 c.c. In the third part of the table the pulse pressure is progressively increased while the aortic resistance is so adjusted as to maintain the coronary outflow constant. It can be seen that the mean aortic pressure had to be dropped by 4 mm. Hg in the case of the largest systemic output. If, in this case, the mean aortic

pressure had been maintained constant, the coronary blood flow would have been a few cubic centimetres larger. For instance, when after the last observation recorded in Table I (c) the mean pressure was increased from 95 to 99 mm. Hg the coronary blood flow increased from 57 c.c. to 64 c.c. per min.

Registration by the hot-wire method (5, 6, 7) shows that the coronary blood vessels are supplied with blood during the whole period of diastole, and that therefore all changes of pressure occurring after the beginning of relaxation of the ventricles must affect the coronary blood flow. The coronary blood flow through the left ventricle is to all evidence completely arrested during systole. It is highly probable, however, that the coronary supply to the right ventricle is not stopped by its less powerful contraction, and if this be the case then it should be expected that not only the post-dicrotic changes in the blood-pressure but also the changes occurring during systole must affect the coronary blood flow. These complex relations are rendered still more difficult of analysis by the fact that the higher the maximum value of the blood-pressure the greater is its effect upon the coronary circulation, and also by the fact that the length of systole and the rate of relaxation of the heart change with alterations in

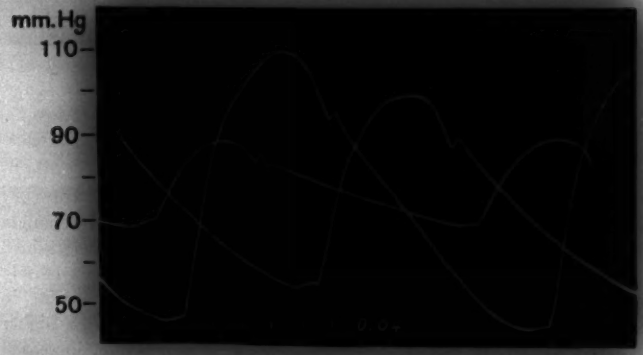

Fig. 1. Record of the aortic blood-pressure taken at different systemic outputs and constant coronary blood flows. The average arithmetical pressure is nearly the same in the three cases, while the diastolic pressure is widely different. This fig. shows the method of retracing used in the calculation of the pressures.

the output. On account of these complications it is not possible, at present, to evaluate more precisely the significance of changes in the

aortic pressure for the maintenance of the coronary blood flow. Calculating by graphic methods from the areas of the curves the true mean pressures prevailing during the whole cycle or during the period of diastole, we found that these gave no more definite relations to the coronary blood flow than the arithmetical mean pressure. The latter can be, therefore, taken as the one determining the coronary blood flow with such accuracy as our present methods allow. Fig. 1 gives a tracing of the aortic pressure as recorded in one of the experiments; Figs. 2 and 3 are redrawn superimposed tracings of the aortic pressure.

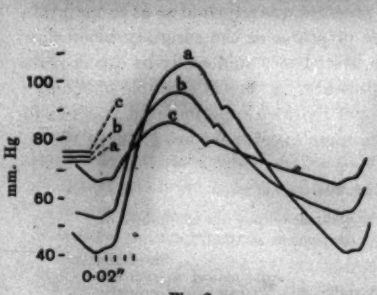

Fig. 2.

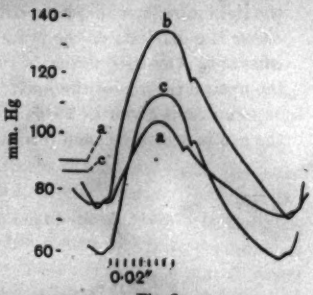

Fig. 3.

Fig. 2. Redrawn superimposed tracings of the aortic blood-pressure. The systemic output in *a*, *b*, and *c* was 960, 520, and 250 per min. respectively. The blood flow from the coronary sinus was constant at 37·5 c.c. per min. The arithmetical average pressures are 73, 74·5 and 76 mm. Hg in *a* to *c* respectively.

Fig. 3. Redrawn tracings of the aortic blood-pressure:

a systemic output	300 c.c.,	coronary sinus blood flow	41 c.c.
b	,, 930 c.c.	,,	,,	115 c.c.
c	,, 950 c.c.	,,	,,	41 c.c.

The diastolic pressure is maintained constant in *a* and *b*. The coronary blood flow is maintained constant in *a* and *c*.

The Effect of Changes in the Heart Rates.

It has been reported on several occasions (1, 2, 3) that in the heart-lung preparation changes in the heart rate have no effect upon the coronary blood flow. Drury (8) has made similar observations in his experiments upon the tortoise heart and Hammouda and Kinosita (9) upon the perfused rabbit's heart. In a recent preliminary communication Miller, Smith and Graber (10) report that in experiments in which a rapid and regular action of the heart followed the stimulation of the auricle the rate of coronary flow was usually increased, while irregular action with

premature contractions produced by stimulation did not cause any significant changes in the rate of coronary flow. These workers do not state whether any precautions were taken to maintain the aortic pressure constant; a condition which assumes an especially great importance since their experiments were performed on the whole animal. In view of the stress which these authors place upon the importance of regularity of the heart beat we decided to repeat the experiments upon the effect of heart rate on the coronary blood flow. The experiments were similar to those upon the effect of output. The heart rate was altered by stimulation of the right auricle, while the aortic pressure was adjusted so as to maintain either the diastolic or the systolic pressures or the coronary blood flow constant. Any irregularity of the heart beat could easily be detected on the optical records of the aortic pressure. A typical example of such an experiment is given in Table II, and redrawn superimposed tracings of the aortic pressure taken from the same experiment are given in Fig. 4.

TABLE II.

Dog—9·5 kl.; heart—58 grm. The systemic output was maintained at 650 c.c. per min. and the temperature at 36·5° C.

Heart rate per min.	Diastolic pressure in mm. Hg	Systolic pressure in mm. Hg	Arithmetical mean pressure in mm. Hg	Blood flow from the coronary sinus in c.c. per min.
(a) The diastolic pressure is maintained constant.				
185	74	88	81	33
150	74	97	85½	38
125	74	110	92	43
103*	74	125	99½	47
(b) The systolic pressure is maintained constant.				
100*	74	125	99½	47
125	80	125	102½	51
150	94	125	109½	57
185	105	125	115	64
(c) The coronary blood flow is maintained constant.				
100*	74	125	99½	47·0
125	79	122	100½	48·5
150	85	117	101	47·5
185	92	113	102½	46·5

* Natural heart rate; the auricle is not stimulated.

The results of the experiments upon the effect of heart rate are similar to those upon the effect of output. The relation of the coronary blood flow to the diastolic and the systolic pressures is the same in both cases; the coronary blood flow follows the changes in the mean aortic pressure,

independently of whether the latter is changed by alteration of the systolic or the diastolic pressures. In order to obtain a constant coronary

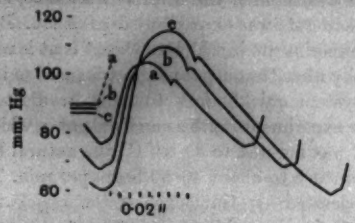

Fig. 4. The aortic pressure is taken from an experiment in which the heart rate was maintained at 125, 150 and 188 beats per min., while the coronary blood flow was kept constant at 39 c.c. by adjusting the arterial resistance.

blood flow at heart rates varying from 100 to 185 beats per min. the mean pressure has to be maintained constant within a few mm. Hg. The slight increase in the mean pressure which can be seen in Table II (c) has been observed in almost every experiment. It is probably due to the fact that the total time occupied by the number of systole during 1 min. is longer at the faster heart rates. In the experiment of Table II the ejection time per min. at the rate of 100 beats was 20·8 sec., while at the rate of 185 beats it was 25·2 sec.

It must be remembered that the term "mean pressure" represents the arithmetical average between the maximal and the minimal pressure. It is a convenient but often incorrect expression of the summated effects of changes in the aortic pressure occurring within a cardiac cycle. In the heart-lung preparation the diastolic aortic pressure cannot fall rapidly below the externally applied constant pressure which determines the arterial resistance. When the aortic pressure becomes equal to that of the external resistance the outflow of blood is possible only through the coronary arteries. This means that when the heart rate is slow and the output per beat small, there will be a longer or shorter period towards the end of diastole during which the aortic pressure will remain nearly constant at values not far removed from the diastolic pressure. This prolonged maintenance of pressure at low values will not show itself in calculation of the arithmetical average pressure, but will obviously influence the calculation of the true mean pressure on which the coronary circulation ultimately depends. The discrepancy between the arithmetical and the true mean pressure will be greater the slower the heart rate or

the smaller the output. This effect can be neglected in the experiments so far described in which the aortic pressure never became equal to the external pressure determining the arterial resistance, since neither the output nor the heart rate was excessively diminished. But it does come into evidence in experiments in which the heart beat is reduced to a very slow rate, especially when the output per beat is small so that the pressure in the arterial system quickly falls towards its diastolic value. For example, we have experiments with a small output in which the temperature of the blood was lowered to 33–34° C., the natural heart rate being reduced in consequence to about 40–50 beats per min. The heart could be, however, accelerated by stimulation of the auricle to 90–115 beats per min. In this case, in order to maintain the coronary blood flow constant the arithmetical mean aortic pressure had to be raised at the slower heart rates by a few millimetres. On inspecting the aortic pressure curves it becomes evident that this apparent influence of heart rate is not due to a direct effect but to the fact that the arithmetical mean pressure ceases to give an approximate representation of the true mean pressure prevailing in the aorta. On calculating the true mean pressure it was found to be nearly constant in conditions in which the coronary blood flow was constant (Table III).

TABLE III.

Dog—9 kl.; heart—52 grm.; Output—200 c.c. per min.; Temperature 33° C.
The coronary blood flow is maintained constant.

Heart rate per min.	Diastolic pressure in mm. Hg	Systolic pressure in mm. Hg	Arithmetical mean pressure in mm. Hg	True mean pressure in mm. Hg	Coronary flow from sinus in c.c. per min.
55*	60	140	100	86	39·0
75	66	118	92	86	40·0
100	70	104	87	87	39·5

* Natural heart rate.

The result of the experiments upon the effect of output and of heart rate on the coronary blood flow shows that changes in the strength of contraction of the heart as well as changes in the heart rate have no direct influence upon the coronary circulation. The changes in the coronary blood flow which may be observed under these conditions are brought about indirectly through alteration of the aortic blood-pressure.

CONCLUSIONS.

1. The coronary blood flow is not determined by either the diastolic or the systolic aortic blood-pressure singly.

2. The coronary blood flow closely follows the changes in the arithmetical average of the systolic and diastolic aortic pressures, which is in most cases a sufficiently accurate measure of the true mean pressure.

3. Changes in the systemic output and changes of the heart rate have within wide limits no direct effect upon the coronary blood flow.

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THE EFFECT OF ATROPINE, ERGOTAMINE, AND PITUITRIN ON PHLORHIZIN GLYCOSURIA.

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It has been suggested in several recent papers that phlorhizin produces glycosuria by acting on the sympathetic nerve endings in the kidney. This suggestion is based on observations of the effect on phlorhizin glycosuria of drugs that paralyse the sympathetic nerve endings. Thus Teschendorf(1) reports a decrease in sugar excretion after the administration of ergotamine to phlorhizinised rabbits. Beck(2) finds a decreased excretion of sugar after the administration of ergotamine to children treated with phlorhizin. Brogsitter and Dreyfus(3) report that the sugar excretion of phlorhizinised animals was inhibited by atropine.

In all these cases the doses of phlorhizin given were small, and the animals were never fully phlorhizinised. In view of the proved decrease in the secretion of urine produced by the drugs atropine and ergotamine, a decrease in the excretion of sugar caused by these drugs under the conditions described is not sufficient proof of an action antagonistic to that of phlorhizin. Some better standard of comparison is required. Such a standard is provided in the constant D/N ratio in the urine of completely phlorhizinised animals on a carbohydrate free diet. For it is reasonable to suppose that if the action of the drug is directly antagonistic to that of phlorhizin, the D/N ratio will fall and less sugar will be excreted, whereas if the drug only decreases the secretion of urine, less sugar will be excreted, but at the same time the excretion of nitrogen will fall and the D/N ratio will be unchanged.

In order to investigate the action of atropine and ergotamine on these lines, rats were phlorhizinised until a constant D/N ratio was obtained, and then the drug was administered. The rat was chosen as the experimental animal partly for convenience in handling, and also because the technique for establishing a constant D/N ratio in the rat had already been worked out in connection with previous experiments not reported here.

The animals were phlorhizinised by the method given by Coolen(4)

for dogs; 50 mg. of phlorhizin suspended in olive oil was injected subcutaneously every day. A satisfactory synthetic carbohydrate free diet was found to consist of protein 75 p.c., fat 20 p.c., salts 5 p.c. On this diet a constant D/N ratio was established after three or four days, the level varying slightly with individual rats. The urine was collected free from faeces in the usual manner over 24 hr. periods. Total nitrogen was determined by Kjeldahl's method after removing any protein contamination from spilt food by precipitating with trichloracetic acid and filtering. The reducing power was estimated by the method of Wood-Ost after precipitating any contaminating protein with metaphosphoric acid. Occasionally the urine was contaminated with a very little food.

Atropine.

Atropine in the form of the sulphate was dissolved in normal saline and administered by subcutaneous injection. The results are given in Table I. All the animals were phlorhizinised for three or four days before the first day given in the table, which is the first day on which a constant D/N ratio was established.

Table I.

Rat and weight before expt.	Day No.	Urine vol. c.c.	Glucose % gm.	Glucose total gm.	Nitrogen per c.c. mg.	Nitrogen total mg.	D/N	Remarks
EF	1	28	5·31	1·47	16·68	467	3·2	
201 gm.	2	52	6·27	3·26	19·51	1015	3·2	
	3	41	6·33	2·59	18·95	777	3·3	
	4	31	6·23	1·93	18·27	566	3·4	5 mg. atropine sulphate
	5	44	5·16	2·27	15·48	681	3·3	
	6	38	7·17	2·72	21·67	823	3·3	
EA	1	60	4·14	2·48	16·00	960	2·6	
230 gm.	2	57	4·73	2·70	18·20	1037	2·6	
	3	22	7·23	1·59	22·06	485	3·3	25 mg. atropine sulphate
	4	11	8·40	0·92	23·71	261	3·5	
	5	40	4·98	1·99	18·38	735	2·7	
EC	1	38	6·30	2·39	20·42	776	3·1	
258 gm.	2	40	7·23	2·89	22·69	908	3·2	
	3	27	7·97	2·05	20·92	565	3·8	50 mg. atropine sulphate.
	4	(6)	5·40	(0·32)	7·84	(47)	6·9	Rat died
EB	1	(41)	5·93	(2·43)	19·47	(798)	3·05	
252 gm.	2	48	6·17	2·96	18·79	902	3·3	
	3	39	6·76	2·64	20·65	805	3·3	
	4	21	7·15	1·50	19·51	410	3·7	100 mg. atropine sulphate.
	5	(11)	6·11	(0·67)	10·52	(116)	5·8	Rat died

Brackets indicate that the sample does not represent a collection over the full 24 hours.

In the above table the D/N ratios are calculated from the figures given for the nitrogen per c.c., and the glucose per 100 c.c. The doses of atropine are recorded opposite the first sample of urine to be collected after injection of the drug, that is opposite the day on which the first effects of the drug might be observed. The 24 hr. samples of urine were collected

from 9.30 a.m. and the atropine injected at 6 p.m., *e.g.* in the case of rat *EF* the figures opposite the fourth day concern the sample of urine collected between 9.30 a.m. on the third day and 9.30 a.m. on the fourth day, and the atropine was injected at 6 p.m. on the third day.

The figures for the D/N ratio show in all cases a rise after atropine, though in the case of rat *EF* the rise after 5 mg. of atropine was negligible. There was a fall in the quantities of urine, total nitrogen, and total glucose. In the case of rats *EC* and *EB* doses of 50 and 100 mg. of atropine respectively were fatal, the animal dying within 48 hours of the injection. According to Clark(5) the toxic dose of atropine for rats varies from 500 mg. per kilogram for young animals to 1·0 gm. per kilogram for adult animals. If Clark's doses are of general application phlorhizinisation appears to lower the resistance of the animal to atropine.

Ergotamine.

Ergotamine in the form of tyramine acid phosphate was dissolved in normal saline and administered by subcutaneous injection. The results are given in Table II. The remarks on the methods of experiment and estimation which were made under the heading of atropine apply to these experiments.

Table II.

Rat and weight before expt.	Day No.	Urine vol. c.c.	Glucose % gm.	Glucose total gm.	Nitrogen per c.c. mg.	Nitrogen total mg.	D/N	Dose of tyramine acid phosphate
EG	1	38	5·43	2·06	17·47	664	3·11	
176 gm.	2	41	5·76	2·36	19·18	786	3·00	
	3	43	5·37	2·31	18·83	810	2·85	1 mg.
	4	39	5·70	2·22	19·29	752	2·95	
	5	38	5·70	2·17	19·29	733	2·95	
	6	35	6·00	2·10	18·72	655	3·20	5 mg.
	7	42	6·24	2·62	19·40	815	3·22	
	8	27	7·77	2·10	23·83	643	3·26	
	9	43	6·49	2·79	20·88	898	3·11	10 mg.
	10	52	5·88	3·06	19·79	1029	2·97	
EH	1	20	5·25	1·05	16·91	338	3·10	
157 gm.	2	33	5·10	1·68	17·52	578	2·91	
	3	31	6·00	1·86	21·79	675	2·75	
	4	26	6·45	1·68	22·01	572	2·93	
	5 i	18	4·77	0·86	14·80	266	3·22	40 mg. in four doses at three hour intervals
	5 ii	12	5·43	0·65	20·08	241	2·70	
	6	31	5·99	1·86	22·35	648	2·71	
	7	34	6·83	2·32	23·19	788	2·95	
EJ	1	35	6·21	2·17	22·39	784	2·77	
170 gm.	2	29	6·84	1·98	22·35	648	3·06	
	3	38	6·99	2·66	21·44	815	3·26	
	4	32	7·43	2·38	22·47	719	3·31	100 mg.
	5	41	6·87	2·82	22·35	916	3·06	

Doses of 1, 5, and 10 mg. of tyramine acid phosphate appeared to have very little action in rat *EG* and the D/N ratio was hardly affected; the

very slight drop in D/N with a dose of 1 mg. is counterbalanced by a slight rise with a dose of 5 mg. In the case of rat *EH*, on the fifth day the urine was collected in two twelve hour periods; in the first period 40 mg. of ergotamine were given in 10 mg. doses at three hour intervals; this dose caused a slight rise in D/N for the first period with a corresponding fall in the second, so that the average for the two periods shows no change. A dose of 100 mg. given to rat *EJ* produced a similar slight rise followed by a slight fall for the next day.

Pituitrin.

No account of experiments on the action of pituitrin on phlorhizin glycosuria occurs in the literature. This is a matter for some surprise in [illegible] the excretion of the urine, *e.g.*

before expt.	Day No.	vol. c.c.	% gm.	total gm.	per c.c. mg.	mg.	D/N	Remarks
			On protein-fat diet; phlorhizin 50 mg. in oil daily					
EH	1	31	5·99	1·86	22·35	648	2·71	
157 gm.	2	34	6·83	2·32	22·19	788	2·95	
	3	31	6·48	2·01	22·01	682	2·94	Pituitrin 0·3 c.c.
	4	35	6·51	2·28	22·01	770	2·96	
			On stock diet; protein-carbohydrate-fat; phlorhizin 60 mg. in Na_2CO_3 daily					
PA	1	15	4·6	0·69	18·2	273	—	
183 gm.	2	17·5	3·4	0·595	14·1	247	—	
	3	21	5·6	1·176	12·5	262	—	
	4	14	6·2	0·868	18·4	257	—	Pituitrin 0·2 c.c.
	5	9	6·7	0·603	18·1	162	—	Pituitrin 0·3 c.c.

Rat *EH* on a protein-fat diet with a constant D/N ratio showed no alteration when injected with 0·3 c.c. pituitrin. In the case of rat *PA*, which was receiving the stock diet and 60 mg. of phlorhizin in Na_2CO_3 daily, the injection of 0·2 c.c. and 0·3 c.c. pituitrin on the fourth and fifth days respectively caused a fall in the volume of urine and in the

from 9.30 a.m. and the atropine injected at 6 p.m., *e.g.* in the case of rat *EF* the figures opposite the fourth day concern the sample of urine collected between 9.30 a.m. on the third day and 9.30 a.m. on the fourth day, and the atropine was injected at 6 p.m. on the third day.

The figures for the D/N ratio show in all cases a rise after atropine, though in the case of rat *EF* the rise after 5 mg. of atropine was negligible. There was a fall in the quantities of urine, total nitrogen, and total glucose. In the case of rats *EC* and *EB* doses of 50 and 100 mg. of atropine respectively were fatal, the animal dying within 48 hours of the injection. According to Clark(5) the toxic dose of atropine for rats varies from 500 mg. per kilogram for young animals to 1·0 gm. per kilogram for adult animals. If Clark's doses are of general application phlorhizini-

For Insertion in Vol. 64.

Erratum. Vol. 64, p. 352.

In the experiments here reported tyramine by misapprehension was used as stated in lieu of ergotamin. Experiments in which the action of ergotoxin was tested are reported in a later paper, this Journ. vol. 65, p. 456.

	5	38	5·70	2·17	19·29	733	2·95	
	6	35	6·00	2·10	18·72	655	3·20	5 mg.
	7	42	6·24	2·62	19·40	815	3·22	
	8	27	7·77	2·10	23·83	643	3·26	
	9	43	6·49	2·79	20·88	898	3·11	10 mg.
	10	52	5·88	3·06	19·79	1029	2·97	
EH	1	20	5·25	1·05	16·91	338	3·10	
157 gm.	2	33	5·10	1·68	17·52	578	2·91	
	3	31	6·00	1·86	21·79	675	2·75	
	4	26	6·45	1·68	22·01	572	2·93	
	5 i	18	4·77	0·86	14·80	266	3·22	40 mg. in four doses at three hour intervals
	5 ii	12	5·43	0·65	20·06	241	2·70	
	6	31	5·99	1·86	22·35	648	2·71	
	7	34	6·83	2·32	23·19	788	2·95	
EJ	1	35	6·21	2·17	22·39	784	2·77	
170 gm.	2	29	6·84	1·98	22·35	648	3·06	
	3	38	6·99	2·66	21·44	815	3·26	
	4	32	7·43	2·38	22·47	719	3·31	100 mg.
	5	41	6·87	2·82	22·35	916	3·08	

Doses of 1, 5, and 10 mg. of tyramine acid phosphate appeared to have very little action in rat *EG* and the D/N ratio was hardly affected; the

very slight drop in D/N with a dose of 1 mg. is counterbalanced by a slight rise with a dose of 5 mg. In the case of rat *EH*, on the fifth day the urine was collected in two twelve hour periods; in the first period 40 mg. of ergotamine were given in 10 mg. doses at three-hour intervals; this dose caused a slight rise in D/N for the first period with a corresponding fall in the second, so that the average for the two periods shows no change. A dose of 100 mg. given to rat *EJ* produced a similar slight rise followed by a slight fall for the next day.

Pituitrin.

No account of experiments on the action of pituitrin on phlorhizin glycosuria occurs in the literature. This is a matter for some surprise in view of the striking effects of pituitrin on the secretion of the urine, *e.g.* the well-known effect of pituitrin in diminishing the diuresis after excessive water-drinking or in diabetes insipidus. We have investigated the effect of pituitrin on phlorhizin glycosuria by two different methods: in the first the drug was administered to the completely phlorhizinised animal with a constant D/N ratio, as in the experiments with atropine and ergotamine, and in the second to the animal on a complete diet (that is, a stock diet of protein, fat, carbohydrate) with daily injections of phlorhizin in 0·25 p.c. sodium carbonate solution. The sterile standardised solutions of pituitrin prepared by Parke Davis and Co. were used in these experiments. Some of the results obtained are given in Table III.

Table III.

Rat and weight before expt.	Day No.	Urine vol. c.c.	Glucose % gm.	Glucose total gm.	Nitrogen per c.c. mg.	Nitrogen total mg.	D/N	Remarks
On protein-fat diet; phlorhizin 50 mg. in oil daily								
EH	1	31	5·99	1·86	22·35	648	2·71	
157 gm.	2	34	6·83	2·32	22·19	788	2·95	
	3	31	6·48	2·01	22·01	682	2·94	Pituitrin 0·3 c.c.
	4	35	6·51	2·28	22·01	770	2·96	
On stock diet; protein-carbohydrate-fat; phlorhizin 60 mg. in Na_2CO_3 daily								
PA	1	15	4·6	0·69	18·2	273	—	
183 gm.	2	17·5	3·4	0·595	14·1	247	—	
	3	21	5·6	1·176	12·5	262	—	
	4	14	6·2	0·868	18·4	257	—	Pituitrin 0·2 c.c.
	5	9	6·7	0·603	18·1	162	—	Pituitrin 0·3 c.c.

Rat *EH* on a protein-fat diet with a constant D/N ratio showed no alteration when injected with 0·3 c.c. pituitrin. In the case of rat *PA*, which was receiving the stock diet and 60 mg. of phlorhizin in Na_2CO_3 daily, the injection of 0·2 c.c. and 0·3 c.c. pituitrin on the fourth and fifth days respectively caused a fall in the volume of urine and in the

total nitrogen, while the percentage of sugar in the urine rose. The total sugar was not affected to any great extent. As there were large variations in the total daily excretion of sugar before the injection of pituitrin, no very strict comparison of the total sugars before and after pituitrin could be made.

Discussion.

Judging from the D/N ratio in the above experiments, atropine has no action antagonistic to that of phlorhizin. This is contrary to the findings of Brogsitter and Dreyfus mentioned earlier. These authors injected atropine and phlorhizin into rabbits and observed the sugar excretion. There was a complete stoppage of urine for two to three hours and the first sample collected after the stoppage always contained sugar, often in greater amounts than the corresponding sample from the control animal receiving phlorhizin alone. They then gave urea as a diuretic to overcome the anuria caused by atropine, and obtained a larger excretion of sugar which was, however, less than the amount excreted by the control animal. The restricted secretion of urine in all these experiments might be sufficient to explain the lessened excretion of sugar after phlorhizin, because with the diminished secretion some of the phlorhizin which would normally pass through the kidney, producing its effect on the cells, might be excreted or destroyed in some other part of the body; again, the increased secretion of urine produced by urea after atropine is not comparable with the secretion of the same amount of urine by the normal kidney.

In 1911 Gargiulo(6) reported that atropine arrested phlorhizin glycosuria in the rabbit but not in the frog; this also suggests that the action of atropine in checking sugar excretion is merely a consequence of the arrest of the kidney function.

That atropine may have some effect on carbohydrate metabolism appears from the finding of Rafael(7) that glycosuria may be a consequence of atropine administration in normal animals. Some such effect may be involved in the rise in D/N ratio observed after atropine in our experiments.

Turning to ergotamine, similar objections may be raised to the experiments of Teschendorf (*loc. cit.*), who admits that there was a large decrease in water excretion in his experiments. He argues that since the decrease in sugar excretion is much greater than the decrease in water elimination, it does not appear probable that the diminution in sugar excretion was due to the decreased water excretion. In all our

experiments the D/N ratio was increased if anything after the administration of ergotamine, and there is no evidence of a decrease in the sugar excretion. The experiments of Adlersberg and Roth(8) may be quoted in this connection; they found that the glycocholia produced by phlorhizin was not affected by ergotamine.

The combined evidence of our experiments with atropine and ergotamine contradicts the theory that phlorhizin causes glycosuria by acting on the sympathetic nerve endings of the kidney.

Summary.

1. Atropine and ergotamine are found to have no definite effect on the D/N ratio in fully phlorhizinised rats on a protein-fat diet.

2. No evidence is obtained of any action of pituitrin on phlorhizin glycosuria in rats.

3. The evidence from the experiments with atropine and ergotamine does not support the theory that phlorhizin produces glycosuria by acting on the sympathetic nerve endings of the kidney.

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A DOUBLE PERFUSION-PUMP.

By H. H. DALE and E. H. J. SCHUSTER.

(*From the National Institute for Medical Research, Hampstead.*)

The heart-lung preparation of Starling and his co-workers has provided a means, extensively used, of studying the functions of the mammalian heart when working against an artificial and adjustable peripheral resistance, which replaces the natural resistance of the systemic vascular system. Many schemes have been devised and used, on the other hand, in which the blood has been perfused through the living vessels of an organ or part of the body by use of an artificial pump. Our original object in devising the pump here described was to produce an adjustable mechanism which could be used to replace the heart, and to carry on both major and minor circulations of the whole body. This would obviously require two synchronously working pumps. Since the two pumps would be working against widely different resistances it was further important that their throws should be rapidly and independently adjustable while running, so as to maintain such equality of output on the two sides as would prevent accumulation of blood on one side of the system, with deleterious back-pressure on the capillaries of the lungs or of the rest of the body, as the case might be. If the pumps and tubular connections were perfectly non-distensible, it should, theoretically, be possible to dispense with such adjustment; the two pumps being set to deliver equal amounts per stroke, they should continue to do this whatever changes occurred in the relation of the pressures they had to overcome. In practice, however, it would be very difficult to make a system of such ideal rigidity. The apparatus which we designed, in which diaphragm-pumps were used, certainly would not have it. The arrangement was accordingly devised which enabled the throw of either pump lever to be increased or decreased quickly and adjusted with delicacy. Embley and Martin (This *Journal*, 32. p. 147. 1905) used two rubber bulb-syringes, compressed by adjustable rotating cams, for a similar purpose. Our apparatus should give greater rapidity and precision of control, but the principle is the same.

Description of the Double Perfusion-Pump.

The pump is shown in plan in Fig. 1 and in elevation in Fig. 2. Both drawings are sectional where this method of presentation has been considered necessary to demonstrate the relations of the working parts. The

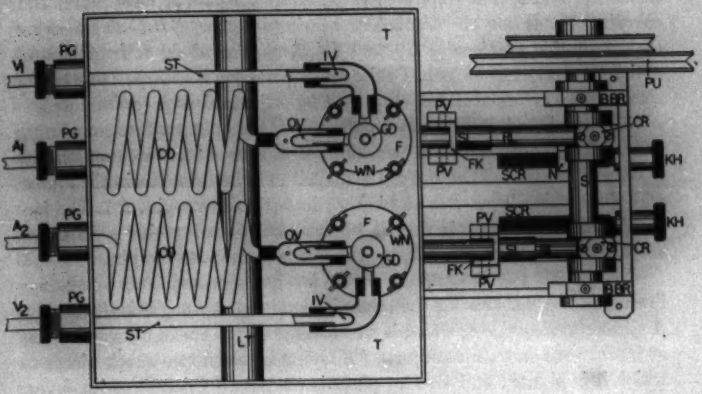
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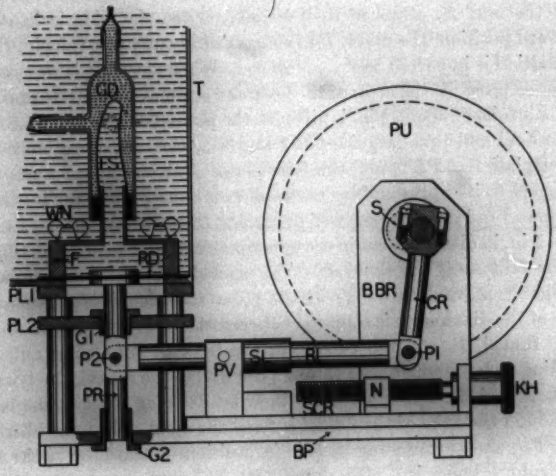
Fig. 1.


T
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CR
PL1
PL2
GT
P2
PV
SL
RL
PI
KH
PR
N
SCR
G2
BP

Fig. 2.

whole apparatus is not shown in Fig. 2. It consists of base plate, *BP*, carrying at one end the crankshaft bearing-bracket, *BBR*, at the other end, a double platform *PL* 1 and *PL* 2, supported on four pillars, of which two only can be seen. To the upper platform is attached one end of a tank, *T*. The other end is supported by adjustable legs (not shown).

The crankshaft is driven by a pulley, *PU*, and operates two connecting rods, *CR* (only one shown in elevation), by means of separate cranks. The lower end of each connecting rod is shaped to fit in a slot cut in the hinder end of a rocking lever, *RL*, and is kept in position by a pin, *P* 1. The forward end of the lever is slotted to form a fork embracing a flattened section of the pump rod, *PR*, to which it is pinned by the pin, *P* 2.

The position of the fulcrum of the lever can be adjusted by the following device, either while the pump is in motion or when it is stationary. A gunmetal sleeve, *SL*, which is free to slide on the rocking lever, carries at its forward end a square fork, *FK*, lying between two cheek pieces to which it is pivoted at *PV*. This pivot is the actual fulcrum of the lever. The cheek pieces are attached below to a slide which runs in a dovetailed guide on the base plate. The position of the slide is adjusted by means of a screw, *SCR* (which can be rotated by its knurled head, *KH*), and the nut, *N*, attached to the slide. When the slide is in its most forward position, the pivot, *PV*, comes exactly opposite the pin, *P* 2, and no motion is imparted to the pump rod. When the slide is screwed back as far as possible, the arms of the lever are approximately of equal length and a stroke of about $\frac{5}{8}$ in. is given to the pump rod. The pump rod, *PR*, is vertical and runs in guides, *G* 1 and *G* 2, carried respectively by the lower platform, *PL* 2, and the base plate. A rubber diaphragm, *RD*, is attached to its upper end by means of two brass plates, one fixed to the pump rod and the other screwed to the first through the rubber. A circular hole, 2 in. in diameter, cut in the upper platform and a corresponding hole in the bottom of the tank allow the central portion of the rubber diaphragm to move downward with the pump rod when it is depressed. The edge of the diaphragm is clamped between the large end of a thick-walled brass funnel, *F*, and the bottom of the tank by four wing nuts, *WN*, working on bolts which pass through the wall of the funnel, the bottom of the tank and the upper platform, their heads being fixed to the latter.

Round the tube of the funnel is fixed the open end of a rubber finger stall, *FS*, and surrounding it, held in position on the tube of the funnel by a rubber bung, is a glass dome, *GD*. The funnel and finger stall are filled with water, and the glass dome outside the finger stall with blood or other perfusion fluid. When the diaphragm rises, water is forced into

the finger stall, which expands and drives the blood out of the glass dome. When the diaphragm falls, the reverse process occurs. In order to provide for the passage in each direction of the blood, two tubes are blown to the side of the dome at right angles to one another and to the long axis of the dome. One of these is connected, as shown in section in Fig. 1, to the inlet valve, *IV*, and the other, to the outlet valve, *OV*. The valves themselves are rubber caps each with a transverse cut near its closed end. The inlet valve is slipped over the end of a straight glass tube, *ST*, which passes through the tank and out of it by way of a packed gland, *PG*, which forms a water-tight joint between the tube and the tank. The valve-chamber is a short section of wide glass tube bent at a right angle and fixed with rubber bungs on the one hand to the straight tube, and on the other to one of the side tubes of the dome. The outlet valve is slipped over the other side tube; it is enclosed in a chamber with one wide end attached by a rubber bung to the side tube and the other end narrow. The narrow end is connected by rubber tubing with one end of a coil of glass tubing, *CO*. The coil at its other end passes into a straight tube which emerges from the tank by way of a packed gland. A small vertical branch tube comes off each of the outlet valve-chambers, and vertical tubes are blown on to the top of each dome. These tubes are used in filling the domes, and to allow air to escape. When the apparatus is in use, they are closed by short lengths of rubber tubing and clips.

Though the figure shows the glass warming-coil attached to the outlet (arterial) side of each pump, it will be obvious that it could equally well be attached to the inflow side, and the straight tube to the outflow; and this arrangement was, in fact, used in the perfusions actually carried out.

The tank is filled with water, which is kept at the desired temperature by a long narrow electric glow lamp housed in a brass tube, *LT*, passing across the tank near its bottom and soldered into holes in the sides. This lamp can be slid in and out of the tube by hand for rough adjustment of the temperature, or can be connected with a thermostat working a magnetic relay for finer control.

Use of the Pump.

The pump has not yet been used for its original purpose of producing a complete circulation of the heartless animal. It has been used, however, with success for experiments in which the hind quarters of a cat or dog were perfused by one pump and the lungs by the other, defibrinated blood being used. Open venous reservoirs were used into which the blood from the abdominal cava and the left auricle respectively discharged, and

from each of which it was sucked into the alternative pump, from "systemic" reservoir into "pulmonary" pump and *vice versa*. When the throws of the two pumps are adjusted to the proper relation, the level of the blood is stationary in both venous reservoirs. Any change in the relation of the peripheral resistances, usually occasioned by a rise or fall of the systemic resistance, causes one venous reservoir to fill at the expense of the other, and necessitates a new adjustment of the relation between the throws of the pumps if a new equilibrium is to be produced. A very satisfactory perfusion can be maintained in this way for many hours.

The details of procedure in setting up an experiment of this kind may be given. The quantity of blood required to fill the apparatus shown in Figs. 1 and 2, including the spaces between finger stalls and glass domes, the valve-chambers, the glass coils and the tubes is about 50 c.c. Another 50 c.c. at least is required to provide a small reserve in the venous reservoirs and fill the connecting tubes. At least 100 c.c. of blood, therefore, should be available to fill the system before a perfusion can be begun, and if any but a very small organ is perfused more will be required. It is accordingly easy to perfuse a dog's hind limbs and lungs with its own blood, but for perfusion of a cat's limbs and lungs it is necessary to use one cat to provide the first charge of blood for the apparatus and a second to provide a further quantity of blood as well as the organs for perfusion. The animals were always bled out under anæsthesia with ether, cats from a cannula tied into the abdominal aorta, dogs from a cannula in a carotid artery. The blood was whipped with an indiarubber brush as it was received in a basin, and filtered through muslin, and then through a plug of washed cotton wool. By the time that the bleeding was completed, of the animal which was to furnish the organs for perfusion, an assistant had filled the apparatus with the earlier part of the defibrinated and filtered blood. The next step was to prepare the lungs for perfusion. The animal being killed, the chest was widely opened, and a bulb cannula, with inlet tube and outlet nozzle and side tubes for insertion of a thermometer and for connection with a manometer, was tied by its nozzle into the main pulmonary artery, which had been clamped as near as possible to its bifurcation, opened and washed out with saline. A strong ligature was then tied in the auriculo-ventricular groove, if this had not been done previously, and the ventricles were usually cut away. A wide cannula was tied through a slit in the apex into the appendix of the left auricle. A respiratory cannula was tied into the trachea, and this with the attached lungs, heart and cannulæ was removed from the body, and

transferred to the apparatus. The arterial cannula being carefully filled with blood was then connected by rubber pressure-tubing with one of the arterial outlets of the pump (say A_1 in Fig. 1). The other arterial outlet (A_2) would be connected for the time directly by blood-filled rubber tubing with V_1. The cannula from the left auricle was connected by a rubber tube so as to discharge into the venous reservoir from which, during action of the pump, the blood was sucked by V_2. The connections all being safely made, to the exclusion of bubbles, the artificial respiration-pump was started, and then clips were removed and the circulation-pump set in motion. During circulation of the first 50 c.c. or so through the lungs, the outflowing blood from the left auricle was caught in a dish, whipped and re-filtered, pre-filtered blood from the reserve being added to the reservoir as required during this process. Finally all the blood available was returned to the system, in which it now circulated into the pump through V_2, out through A_2, directly from this to V_1, out through A_1 to the pulmonary artery, through the lungs, and from the left auricle back to the reservoir feeding V_2.

While the defibrinated blood was thus being circulated through the lungs, cannulæ were being inserted for the systemic perfusion, either into the lower ends of the abdominal aorta and vena cava, for perfusion of both hind limbs, or into the femoral artery and vein for perfusion of one leg. In either case, loss of blood through collateral channels had to be excluded by careful mass ligaturing. The hind quarters were then detached from the rest of the carcase and transferred to the table adjoining the perfusion-pump, which was now stopped while the attachments were completed. The arterial cannula was filled and attached, with exclusion of bubbles, to the pressure-tube leading from A_2, while the blood from the venous cannula was led by a rubber connection to discharge into the reservoir feeding V_1. The pump being started, the first portion of blood issuing from the limb-veins was again caught, whipped, re-filtered, and then returned to the reservoir. Connection was now made between the arterial cannulæ and the corresponding manometers and the strokes of the pumps so adjusted that the levels in the two reservoirs remained practically constant.

In our experience, as in that of other workers, the use of the lungs for oxygenation preserves the blood in a much more physiological condition than does an artificial oxygenator, such as that devised by Hooker and used by Burn and Dale. Not only is the potent vaso-constrictor effect of the freshly defibrinated or heparinised blood removed by the preliminary circulation through the lungs, but the moderate and more

normal vascular tone of the systemic vessels thus produced is maintained during a long perfusion; the vessels long retain their reaction to constrictor and dilator drugs, and œdema of the organs is long postponed.

It will be obvious that, where a single pump only, with readily and accurately adjustable throw, is required for any physiological purpose, one pump of the type described above can be used. Such a unit, as made by C. F. Palmer, Ltd., is illustrated in Fig. 3. Obviously two or more of

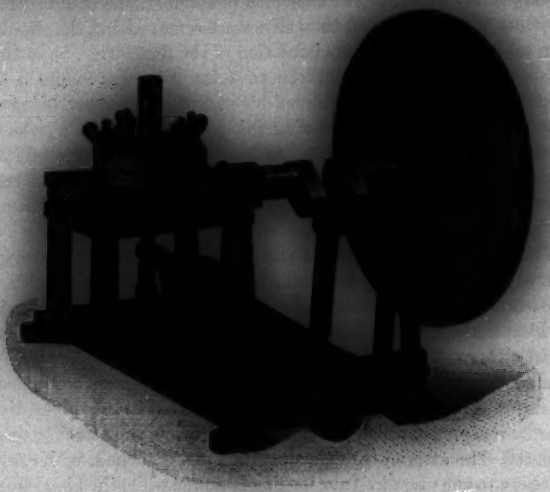

Fig. 3.

these could be arranged to drive from a common shaft. Similarly the thermostat bath, in which the pumps are immersed in the system above described, may for some purposes be omitted or replaced by one more suitable for the particular need. We are having one constructed at present in which the tubes from the pumps enter a chamber jacketed with warm water, in which a double perfusion of liver and lungs can be carried out without surface cooling of those organs. For the perfusion of the liver, as described by Dr Bodo and Mr Marks in a following paper, the use of the apparatus was further modified by using one pump to raise blood to an overflow reservoir, from which the portal vein was perfused under constant hydrostatic pressure, while a pulsatile pressure was produced

Fig. 4. Lines of record, from above downwards: Volume of fluid in "pulmonary" venous reservoir; "systemic" arterial pressure; "pulmonary" arterial pressure; pressure zero; time in 10 sec. intervals.

in the hepatic artery by use of the other pump. The apparatus can doubtless be adapted in other ways for other special purposes.

Fig. 4 shows a record of the pressures in the "pulmonary" and "systemic" arterial cannulæ in perfusion through an artificial scheme, the systemic and pulmonary resistances being represented by a high and a low resistance respectively on the two sides of the double circuit. The volume recorder, communicating with the air-space above the fluid in the "pulmonary" venous reservoir, equivalent to a stationary left auricle, shows (*LA* in Fig. 4) the balance of the circulation through the two widely different resistances produced by adjusting the relation of the two pump-strokes. A slight tightening, at T_1, of the clamp producing the "systemic" resistance causes a rise of systemic arterial pressure from about 80 to 180 mm., and an increase of the volume of fluid in the pulmonary venous reservoir, at the expense of that in the systemic reservoir. A small adjustment of the pump at A_1 restores the balance at the new relation of resistances, but the increase of stroke required to effect this causes the systemic pressure to rise further to about 220 mm. At L_1 the clamp producing the systemic resistance is slightly relaxed, producing a fall of systemic arterial pressure, and a loss of fluid from the pulmonary venous reservoir. To balance this loss, the stroke of the systemic pump must again be reduced at A_2, producing a further fall in the systemic pressure. The whole cycle is then repeated at T_2, A_3, L_2 and A_4. It will be seen that the apparatus is made to produce, after a delay allowing the disturbance of equilibrium to become manifest, that adjustment of the work done by the pump to the resistance encountered by the outflow, which is necessary to restore equality of output from both pumps. This is, of course, the adjustment which the living ventricle makes immediately and with great precision, and failing which any increase of systemic resistance would produce rapid dilatation of the left auricle, and back pressure on the lungs. Apart from its use in carrying out experimental perfusions, and studying the effects of vascular reactions uncomplicated by this automatic adjustment of the living heart, the apparatus might have some value for purposes of demonstration, enabling the nature of this adjustment, and its necessity, to be shown with great clearness and precision.

Summary.

A double perfusion-pump is described, by which any part of the systemic vascular system and the pulmonary system may be perfused in continuity, and the outputs adjusted in relation to the varying resistances while the pump is in motion.

THE EFFECT OF THE "HEART-TONICS" AND OTHER DRUGS UPON THE HEART-TONE AND CORONARY CIRCULATION.

By RICHARD BODO.

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THOUGH there exists a considerable literature dealing with the subject of the heart-tonics, it has never clearly shown the uncomplicated effect of these drugs upon the heart. The majority of these investigations were made on the frog's heart, but under conditions which are never to be found *in vivo*, in that in these experiments the venous pressure and arterial resistance were identical. Since these conditions do not correspond to the physiological conditions the conclusions drawn from these experiments cannot be entirely convincing. Another group of investigators used the mammalian heart as the experimental object and performed their experiments partly on the isolated heart, partly on the whole animal, on hearts left *in situ*. Though the isolated heart is quite a useful preparation for some purposes, it is not suitable for the study of the effect of the heart-tonics, because, the heart chambers being more or less empty, the heart performs much less work than *in vivo*, the resistance being negligible. The experiments performed on the whole animal have shown many very important facts, but in spite of these, they do not show simply the direct effect on the heart, since this might be complicated by the effect produced on the nervous system, which might change the heart rate, with a resulting change in the output. Even after exclusion of nervous effects there is another complicating factor to be dealt with, practically every heart-tonic having an effect on the vessels as well, which might change the venous inflow and ventricular output, thus obscuring the direct effect of the tonic drug on the heart.

For the study of the direct effect of a drug upon the heart, Starling's heart-lung preparation seemed to be the most suitable, because in this preparation all the environmental conditions of the heart, viz., temperature, composition, *p*H, volume and pressure of the entering blood, and the opposition to the outflow offered by the arterial resistance, can be exactly

controlled, changed within very wide limits, or kept unaltered during the whole of the experiment. In this preparation the frequency of the beat depends only on the temperature of the pace-maker, provided that the *p*H of the blood is constant; and as has been shown in previous work, the hydrogen ion concentration of the blood, its CO_2 and lactic acid content do not change appreciably during the course of an experiment after the first quarter of an hour. So that, by keeping the temperature of the entering blood constant, we can be assured of a regular rhythm throughout the whole of the experiment.

Further, it is quite easy to maintain the work of the heart constant, or to alter it within wide limits by changing the inflow or the arterial resistance. In this preparation the direct effect of a drug upon the heart ought to be seen quite clearly. Hitherto, however, no experiments have been performed on the effects of the heart-tonics on the heart-lung preparation, with the exception of one investigation by Bijlsma and Roessingh(1), who used the heart-lung preparation from the cat in order to investigate the effect of Strophanthin. It seemed worth while, accordingly, to investigate more fully the effect of the heart-tonics upon the heart under these conditions.

In the first place clear definitions must be given of the meaning in the present paper of the terms "heart-tone" and "heart-tonics," since the term "tone" has been employed by many investigators in dealing with the heart, both in physiological and pathological literature, with a different implication. Here it will be used as Starling defined it, in one of his earlier papers(2), as "synonymous with physiological condition or fitness of the muscle fibre," and more recently and more exactly(3) as "the mechanical efficiency of the muscle fibre, *i.e.* the relation of the mechanical energy to the total energy liberated." It was found by Starling and Visscher(3) that the oxygen consumption of the heart—which served as a measure in their experiments of the total energy set free in the heart during its activity—provided that the chemical and temperature conditions were maintained constant, was determined by the initial length of its muscular fibres, *i.e.* by its diastolic volume. That means that, if the same work is done by two hearts, in one case with a small diastolic volume and in the other with a large diastolic volume, the mechanical efficiency of the former is greater, the tone of the former is better than that of the latter. This enables us to measure the heart-tone by measuring the diastolic volume, provided that the work performed by the heart is maintained constant during the whole of an experiment. It may accordingly be expected of a drug which is described as heart-tonic,

that it will enable the heart to perform the same work as before with a smaller volume.

Methods. All the experiments have been carried out on dogs; the heart-lung preparation was made in the usual way, as described by Starling(4) and his fellow workers from this Laboratory. The heart rate was maintained constant by keeping the temperature of the heart constant. The temperature was measured in the blood entering the heart. The rate of the beat was recorded on a kymograph by a tambour, which was connected by air transmission with a small piston recorder, the piston of which was pulled by means of a thread by the contracting auricle. The output of the heart and the arterial pressure were maintained constant by keeping the venous inflow and the arterial resistance constant. The output of the left ventricle was determined by measuring the amount of blood flowing out on the venous side of the resistance. This did not include the coronary flow; in order to determine the latter a Morawitz cannula was introduced into the coronary sinus. According to Evans and Starling(3) and Markwalder and Starling(3) this drains only three-fifths of the coronary blood. The arterial pressure was measured by connecting the cannula in the innominate artery by means of a side branch with a mercurial manometer, the oscillations of which were recorded on the kymograph. The amount of blood entering the heart was adjusted to the size of the heart, and it varied from 300–600 c.c. per min. The arterial resistance was adjusted to produce an arterial pressure of 100 mm. Hg. Under these conditions the volume of the heart-lung preparation remains for a time steady, but later, as the heart tires, it dilates continuously. The drug was administered into the venous blood reservoir; in some of the experiments this was done at a time while the heart volume was still normal, but in the majority of the experiments when the heart had become already tired and dilated, since it was thought that the conditions of the dilated heart would correspond more closely with those found in the pathological state. In order to produce the heart dilation quickly at the beginning of an experiment either the venous inflow or the arterial resistance or both were increased. In order to measure the volume of the heart a Henderson cardiometer was employed, fitted with a special rubber sleeve of very thin material, which lay snugly against the heart. The cardiometer was connected by air transmission with Palmer's large piston recorder, the volume change of which was recorded on a kymograph. Special care was taken to make an absolute air-tight seal around the ventricles without compressing the atrio-ventricular ring. To control the pressure in the right auricle—especially when fixing the cardiometer on the heart—a cannula was introduced into the inferior vena cava which was connected with a saline manometer.

Among the heart-tonics, the effects of the following have been investigated: Digitalis, Caffeine, Camphor and Strychnine.

Experiments on the Heart-Tone.

Digitalis. Of the Digitalis group the effects investigated were those of *g*-Strophanthin, of Digitalis tincture and of the Digitalis infusion. In the first experiments *g*-Strophanthin was tested, and it was used in the same dose as was employed by Bijlsma and Roessingh(1) on the cat's heart, namely, 0·03 mgm. *g*-Strophanthin per 100 c.c. blood. In our experiments this dose, however, very soon caused a toxic effect. It was necessary to perform a great number of experiments before the proper dose was found; in the earlier experiments it was either given in too small

a dose, which had no effect, or in too large a dose, which led very quickly to poisoning of the heart. When used in the right dose, which was found in our experiments to be 0·0025 mgm. *g*-Strophanthin per 100 c.c. of blood, it improves the function of the heart. In most experiments the improvement is a real tonic effect, the heart volume becoming smaller, though the work performed by the heart is the same as before, the output, arterial resistance and heart rate being the same. If the improving effect is most marked (optimal), the heart comes back to its original volume (Fig. 1). That the output, even at that smaller heart volume, remains the

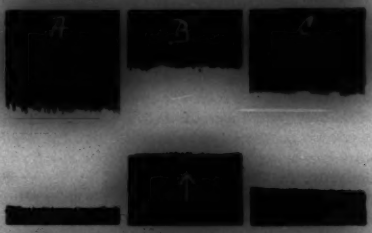

Fig. 1. Strophanthin.

A = heart normal. Temp. 36·0° C. Heart rate = 138. Outflow = 550 c.c. per min. B.P. = 100 mm. Hg. V.P. = 9·5 cm. H_2O. Heart's weight = 88 gm. Amount of blood = 1000 c.c. *B* = heart dilating. At † 0·025 mg. *g*-Strophanthin (0·0025 mg. per 100 c.c. of blood) was added. *C* = 30 min. later. Heart volume diminishing, tonic effect.

same as before, can be observed not only by measuring the amount of blood flowing out on the venous side of the resistance, but it can also be seen from the cardiometer tracing, the difference between the systolic and diastolic volumes, which shows the output, remaining unaltered (Fig. 1).

Sometimes the improvement is only a prevention of further dilatation of the heart, and sometimes there is no improvement at all; possibly in such cases the drug was given too late so that the heart could not react any more. Whatever improvement occurred was not apparent immediately after the administration of the drug; it was manifest only about 15–30 min. later, but it was lasting. Strophanthin in the dose used here did not change the heart rate.

Digitalis tincture produced almost the same effect (Fig. 2), and the effect appeared about the same time after the administration.

It seemed possible that quicker and more regularly definite results

might be obtained by using a Digitalis preparation not containing alcohol like the two already discussed, since it is known from Sulzer's work(7)

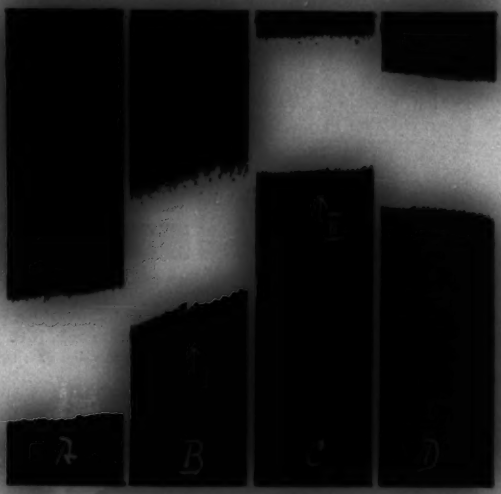

Fig. 2. Digitalis tincture.

A = heart slightly dilating. Temp. 37·0° C. Heart rate = 150. Outflow = 600 c.c. per min. B.P. = 130 mm. Hg. V.P. = 9·5 cm. Heart's weight = 73 gm. *B* = well-marked dilation. At † I. 0·5 c.c. Digitalis tincture was added. *C* = 5 min. later. Heart still further dilating. At † II. 1·0 c.c. Digitalis tincture was added. *D* = 20 min. later, tonic effect.

that alcohol causes a heart dilatation in the heart-lung preparation, and it seemed conceivable that the dilating effect of alcohol might weaken or antagonise the tonic effect of Digitalis. In order to exclude the complicating effect of alcohol, an infusion of Digitalis leaves (in the concentration of 1 : 100) was prepared and used for the experiments; the tonic effect obtained in this way was more marked, the volume decreased in every experiment to normal, but the effect was again produced only after 15–20 min. (Fig. 3.)

The tonic effect caused by all these preparations of Digitalis though delayed in onset was lasting, except when the dose was too large, so that the tonic effect was followed very soon by the toxic effect.

Caffeine. In contrast to the effect of Digitalis, which, as shown above,

is delayed in onset but lasting in character, the tonic effect of Caffeine is rapid in onset but evanescent (Fig. 4). The Caffeine was administered as

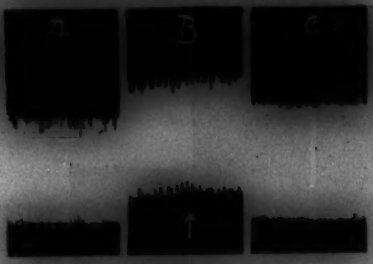

Fig. 3. Digitalis infusion.

A = heart normal. Temp. 37·0° C. Heart rate = 145. Outflow = 571 c.c. per min. B.P. in the beginning = 110 mm. Hg., later = 130 mm. Hg. V.P. = 8·0 cm. H_2O. Heart's weight = 82 gm. *B* = heart dilating. At † 10·0 c.c. Digitalis infusion (1 : 100) were added. *C* = 20 min. later, tonic effect.

the double benzoate of caffeine and sodium, in such a dose (0·05 to 0·1 gm. of caffeine reckoned as base) that the rate of the heart was not affected. When the effect of the Caffeine is over the heart resumes its slow dilatation;

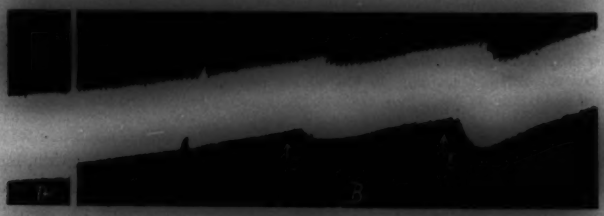

Fig. 4. Caffeine.

A = heart slightly dilating. Temp. 37·0° C. Heart rate = 153. Outflow = 500 c.c. per min. B.P. = 140 mm. Hg. V.P. = 9·5 c.c. Heart's weight = 133 gm. *B* = Heart further dilating. At $\dagger_{I}$. 0·05 gm. Caffeine was added, transitory tonic effect. At $\dagger_{II}$. 0·10 gm. Caffeine was added, transitory tonic effect.

and the effect of Caffeine can be repeated seyeral times, since the tonic effect does not give way to toxic action, as in the case of Digitalis.

Camphor. To our surprise and in contrast to the tonic effects of Digitalis and Caffeine, Camphor caused dilatation of the heart. In the

earliest experiments the alcoholic solution of Camphor was used (0·10 gm.) and produced in every case a well-marked heart dilatation (Fig. 5). The

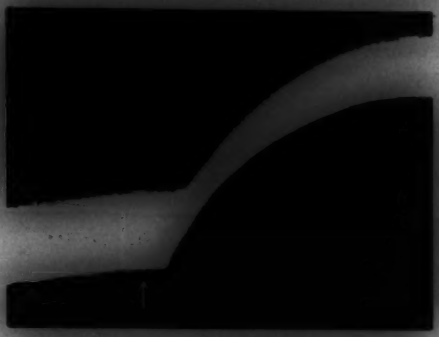

Fig. 5. Camphor (dissolved in Alcohol-Saline).

Temp. 37·0° C. Heart rate = 158. Outflow = 400 c.c. per min. B.P. = 105 mm. Hg. V.P. = 8·0 cm. Heart's weight = 81 gm. Heart dilating. At ↑ 0·10 gm. Camphor dissolved in Alcohol-Saline was added, caused a well-marked dilatation.

result obtained seemed to be in contradiction to the generally accepted medical opinion concerning Camphor, and therefore the possibility that the solvent alcohol might be responsible for the dilatation of the heart had first to be excluded. Camphor oil could not be used for our purpose and the Aqua Camphorata U.S.P. was therefore used as the only suitable preparation. This was prepared according to the prescription of the U.S.P., 0·8 gm. Camphor being triturated with 0·8 c.c. of 95 p.c. Alcohol in a mortar, 1·5 gm. Purified Talc being then added and the trituration continued until the Alcohol evaporated, 100 c.c. recently boiled distilled H_2O being finally added. The mixture was repeatedly filtered until the Camphor Water became perfectly clear. The Camphor content of Camphor Water prepared in this way was not exactly known, because a great amount of the Camphor was retained on the Talc and probably only a very small part went through the filter; 10–20–30 c.c. of this solution were used for the experiments (that is, the average therapeutic dose of this preparation according to the U.S.P.), and these doses produced in every case a very marked dilatation of the heart. In order to make these results quite convincing, there were two factors still to be excluded. Firstly, the large amount of fluid used might be responsible for the dilating effect

obtained (change in the viscosity of the blood?), and secondly—since the Aqua Camphorata still contains traces of Alcohol—the Alcohol present might be the responsible factor. Control experiments were performed for this purpose. 20–30 c.c. Saline were added to the venous reservoir to control the volume-effect, and an Alcohol-Saline solution was prepared in the same way as the Aqua Camphorata U.S.P., containing all the constituents of the Aqua Camphorata except the Camphor, and 20–30 c.c. of this solution were similarly added. These experiments, however, produced only negative results; neither the 20–30 c.c. Saline nor the 20–30 c.c. Alcohol-Saline solution produced any dilatation of the heart (Fig. 6).

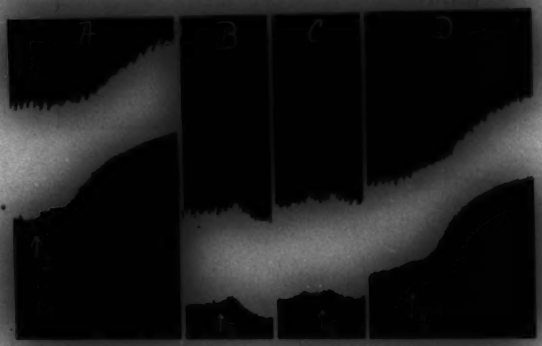

Fig. 6. Aqua Camphorata U.S.P. Saline. Alcohol-Saline.

Temp. 37·0° C. Heart rate 138 per min. Outflow = 430 c.c. per min. B.P. = 110 mm. Hg. V.P. = 7·0 cm. H_2O. Heart's weight = 101 gm. *A* = Heart normal, at ↑ I. 20·0 c.c. Aqua Camphorata U.S.P. added. *B*. At ↑ II. 30·0 c.c. of Saline added. *C*. At ↑ III. 20·0 c.c. Alcohol-Saline (0·8 : 100) added. *D*. At ↑ IV. 20·0 c.c. Aqua Camphorata U.S.P. added.

It is accordingly proved that the dilatation of the heart following administration of Camphor is a genuine effect of that substance. Camphor in the dose used did not alter the heart-rate.

Strychnine. Finally Strychnine was tested in this preparation, because it has been largely used by clinicians on the assumption that this drug has a tonic effect on the human heart. In the heart-lung preparation from the dog no such effect has been seen (Fig. 7).

EFFECTS OF THE ABOVE DRUGS ON THE CORONARY CIRCULATION.

It seemed worth while to investigate the effect of these drugs upon the coronary circulation, because there was the possibility that the tonic

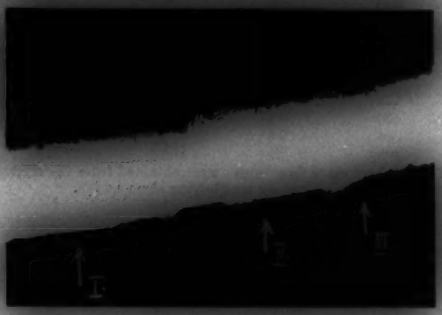

Fig. 7. Strychnine.

Temp. 37·0° C. Heart rate 142 per min. Outflow = 413 c.c. per min. A.P. = 110 mm. Hg. V.P. = 8 cm. H_2O. Heart's weight = 82 gm. At ↑I. 0·0002 gm. Strychnine sulph. added. At ↑II. 0·0004 gm. Strychnine sulph. added. At ↑III. 0·0010 gm. Strychnine sulph. added.

effect was only a consequence of the effect produced on the coronary vessels. It was quite conceivable that a drug might produce its tonic effect by dilating the coronary vessels and thereby improving the blood flow through the heart muscle. On the other hand, there was the possibility that the sequence might be the opposite of this, the primary effect being the action on the heart muscle fibres, a tonic effect causing a compression of the coronary vessels, or a dilating action on the muscle producing a passive widening of the coronary vessels.

The experiments performed for this purpose (Tables I, II, III, IV) show that the Digitalis group (Strophanthin, Digitalis tincture), Caffeine and Camphor all dilate the coronary vessels. The increase in the coronary flow by Strophanthin and Digitalis tincture was only a moderate one, and it did not appear immediately; Caffeine increased the coronary flow much more, and the effect was instantly noticeable. Camphor produced only a slight increase in the coronary flow.

TABLE I.

Temp. 37·0° C. Heart rate = 150 per min. B.P. = 100 mm. Hg.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary* flow (calc.) c.c. per min.	Addition of
1.00	430	51	85	
1.10	—	54	90	
1.15	—	54	90	
1.16	—	—	—	0·1 c.c *g*-Strophanthin
1.18	430	51	85	
1.20	—	51	85	
1.21	—	—	—	0·2 c.c. *g*-Strophanthin
1.23	430	60	100	
1.26	—	60	100	
1.35	—	69	115	

* The total coronary flow figures were obtained from the coronary sinus output by multiplying them by 5/3.

TABLE II.

Temp. 37·0° C. Heart rate = 150 per min. B.P. = 100 mm. Hg.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
3.08	550	32	53	
3.13	—	32	53	
3.14	—	—	—	0·5 c.c. Digitalis tincture
3.16	—	32	53	
3.19	550	32	53	
3.25	—	32	53	
3.27	—	—	—	0·5 c.c. Digitalis tincture
3.29	—	32	53	
3.33	—	—	—	1·0 c.c. Digitalis tincture
3.38	—	—	—	1·0 c.c. Digitalis tincture
3.39	550	40	66	
3.40	—	—	—	6·0 c.c. Digitalis tincture
3.41	—	56	93	
3.44	500	71	118	
3.47	—	72	120	
3.48	—	—	—	Toxic effect

TABLE III.

Temp. 37·0° C. Heart rate = 162 per min. B.P. = 110 mm. Hg.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1.55	375	41	68	
2.05	—	43	72	
2.11	—	43	72	
2.12	—	—	—	0·10 gm. Caffeine
2.13	—	99	165	
2.14	—	108	180	
2.21	261	111	185	
2.22	—	—	—	0·10 gm. Caffeine
2.24	—	135	225	
2.27	240	132	220	

TABLE IV.

Temp. 36·0° C. Heart rate = 138 per min. B.P. = 110 mm. Hg.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1.08	462	36	60	
1.09	—	—	—	1·0 c.c Aqua Camphorata. U.S.P.
1.10	462	36	60	
1.13	462	39	65	
1.14	—	—	—	5·0 c.c. Aqua Camphorata U.S.P.
1.16	—	42	70	
1.18	—	44	73	
1.19	375	47	78	
1.20	—	—	—	20·0 c.c. Aqua Camphorata U.S.P.
1.22	364	60	100	
1.24	—	63	105	
1.26	—	63	105	
1.27	—	—	—	25·0 c.c. Aqua Camphorata U.S.P.
1.28	308	78	130	
1.31	—	81	135	
1.32	—	—	—	25·0 c.c. Aqua Camphorata U.S.P.
1.34	353	90	150	
1.37	—	93	155	

EFFECTS OF CERTAIN OTHER DRUGS.

In order to obtain yet further evidence as to a possible relationship between effects on the heart muscle, on the one hand, and on the coronary circulation on the other, it was thought desirable to examine by the same methods certain other substances which had no reputation as heart-tonics, but were known or suspected to have an action on the coronary vessels.

Nitrites. Though Lauder-Brunton(8) introduced Amyl nitrite in therapeutics in 1867, its vaso-dilator effect on the coronary arteries was only later demonstrated by Cow(9) and Pal(10) on isolated vessel strips, and by Schloss(11) and Loeb(12) on the coronary circulation. Our purpose is to determine whether it would have the same effect in the denervated heart-lung preparation.

As can be seen in Tables V and VI both Amyl nitrite and Sodium nitrite increased the coronary flow also in this preparation, and the effect appears very rapidly and can be repeated many times. With regard to their action on the heart-tone there is a difference between them, Amyl nitrite having no effect at all and Sodium nitrite, in larger doses, causing a heart dilatation (Figs. 8 and 9).

TABLE V.

Temp. 37·0° C. Heart rate = 142 per min. B.P. = 100 mm. Hg.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1.55	444	36	60	
2.00	—	36	60	
2.05	—	36	60	
2.08	469	—	—	
2.11	—	36	60	
2.12	—	—	—	3 minims Amyl nitrite mixed in the inspired air
2.13	—	84	140	
2.15	—	60	100	
2.17	444	45	75	
2.23	—	33	55	
2.24	—	—	—	3 minims Amyl nitrite
2.24	—	96	160	
2.25	—	90	150	
2.26	—	51	85	
	—	60	100	
2.27	—	54	90	
2.28	429	45	75	
2.31	—	45	75	
2.32	—	—	—	3 minims Amyl nitrite
2.32	—	87	145	
2.33	—	66	110	
2.34	—	60	100	
2.35	—	—	—	3 minims Amyl nitrite
2.35	—	72	120	
2.36	—	111	185	
2.37	—	111	185	
2.38	340	105	175	
2.39	—	96	160	
2.40	—	87	145	
2.41	—	78	130	
2.42	—	72	120	
2.43	—	66	110	
2.44	—	72	120	

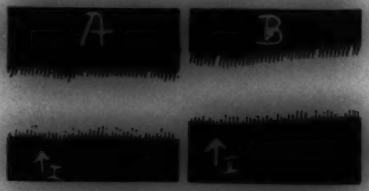

Fig. 8. Amyl nitrite.

A = Heart normal. Temp. 36·0° C. Heart rate = 142 per min. Outflow = 444 c.c. per min. B.P. = 130 mm. Hg. V.P. = 7·0 cm. H_2O. Heart's weight = 79 gm. At ↑I. 3 minims Amyl nitrite mixed with the inspired air. *B* = Heart slightly dilating. At ↑II. 3 minims Amyl nitrite injected into the venous blood close to the heart.

TABLE VI.

Temp. 37·0° C. Heart rate = 148 per min. B.P. = 110 mm. Hg.
Heart's weight = 130·0 gm.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
4.36	444	35	58	
4.41	—	35	58	
4.46	—	35	58	
4.47	—	—	—	0·05 gm. Sodium nitrite
4.49	—	41	68	
4.53	—	45	75	
4.55	444	45	75	
4.56	—	—	—	0·10 gm. Sodium nitrite
4.58	—	54	90	
5.00	—	66	110	
5.01	—	—	—	0·15 gm. Sodium nitrite
5.02	—	71	118	
5.05	—	90	150	
5.06	—	102	170	
5.08	345	114	190	
5.10	—	—	—	0·15 gm. Sodium nitrite
5.13	250	162	270	
5.15	—	180	300	
5.17	—	195	325	
5.19	—	213	355	
5.23	—	240	400	

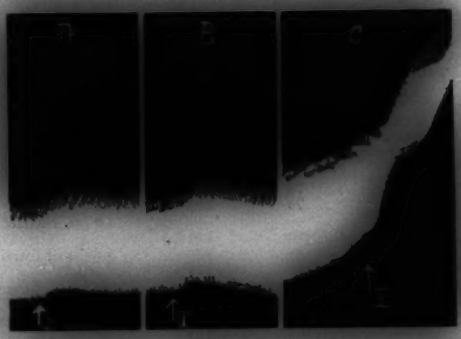

Fig. 9. Sodium nitrite.

A = Heart normal. Temp. 37·0° C. Heart rate = 132 per min. Outflow = 429 c.c. per min. B.P. = 100 mm. Hg. V.P. = 6·0 cm. H_2O. Heart's weight = 89 gm. At ↑ I. 0·10 gm. Sodium nitrite was added. *B*. At ↑ II. 0·15 gm. Sodium nitrite was added. *C*. At ↑ III. 0·50 gm. Sodium nitrite was added, heart dilating.

Insulin. It was recently shown by Visscher and Müller(33) from this Laboratory that Insulin produces a tonic effect, that is to say that, provided the heart rate, the venous inflow and the arterial resistance are maintained constant, the work performed by the heart, and the output remain the same, but the heart volume is diminished under its action. This effect, which lasts very long, was obtained in our experiments with as little as five units of Insulin and could not be repeated, a second dose being always ineffective (Fig. 10). The effect of Insulin was tested on the

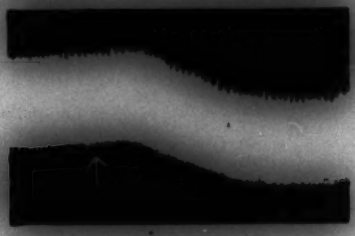

Fig. 10. Insulin.

Temp. 37·0° C. Heart rate = 150 per min. Outflow = 522 c.c. per min. B.P. = 110 mm. Hg. V.P. = 8·0 cm. H_2O. Heart's weight = 86 gm. Before the administration of Insulin 1·0 gm. Dextrose was added. At ↑ 10 units of Insulin were added. Heart volume diminishing, tonic effect.

TABLE VII.

Temp. 37·0° C. Heart rate 152 per min. B.P. = 110 mm. Hg.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1.40	444	75	125	
1.45	—	78	130	
1.50	—	78	130	
1.51	—	—	—	1·0 gm. Dextrose
1.55	—	90	150	
1.56	—	—	—	5 units of Insulin
2.00	444	83	138	
2.05	—	93	155	
2.10	444	93	155	
2.13	—	—	—	10 units of Insulin
2.18	—	66	110	
2.19	—	60	100	
2.23	—	60	100	
2.28	—	66	110	
2.30	—	—	—	10 units of Insulin
2.34	—	54	90	
2.39	—	51	85	
2.46	—	54	90	

coronary circulation (Table VII), and it was found that a small dose (five units), which was sufficient to produce a tonic effect, had either no influence on the coronary circulation or caused a slight transitory constriction, but that a larger dose (ten units) constricted the coronary vessels, diminishing the coronary flow.

Pituitrin. Pituitrin, which has a vaso-constrictor effect on the vessels in general (Oliver and Schäfer(13)), diminished also the coronary flow in Dale's(14) experiment on the isolated heart. This latter effect was observed in this preparation as well, but could be produced only once, not more, even with a larger dose (Table VIII). Its effect on the heart

TABLE VIII.

Temp. 37·0° C. Heart rate = 150 per min. B.P. = 110 mm. Hg. Heart's weight = 89·0 gm.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1.15	444	20	33	
1.25	—	21	35	
1.34	444	23	38	
1.35	—	—	—	0·1 c.c. Pituitrin
1.37	444	17	28	
1.38	—	14	23	
1.39	—	14	23	
1.40	—	14	23	
1.43	444	15	25	
1.47	—	17	28	
1.53	444	22	37	
1.54	—	—	—	0.2 c.c. Pituitrin
1.55	—	21	35	
1.57	—	18	30	
1.59	462	23	38	
2.01	—	24	40	
2.02	—	—	—	0·5 c.c. Pituitrin
2.03	—	24	40	
2.04	462	24	40	
2.06	—	28	47	
2.08	—	30	50	
2.10	—	30	50	
2.12	462	32	53	

volume was also observed and it was found that it dilated the heart immediately after its administration in a very high degree, but that this dilatation was of transitory character and could also be observed only once or, in some of our experiments, twice, but no more; if a second dilatation occurred, it was never so marked as the first (Fig. 11).

As in our experiments the volumes of both ventricles are recorded together and cannot be separated, it was conceivable that in the observed heart dilatation after the administration of Pituitrin the right ventricle played a greater part, for which an increase in the resistance of the

pulmonary system, caused either by the direct constriction of the vessels or by the compression of these vessels by the constricted bronchioli,

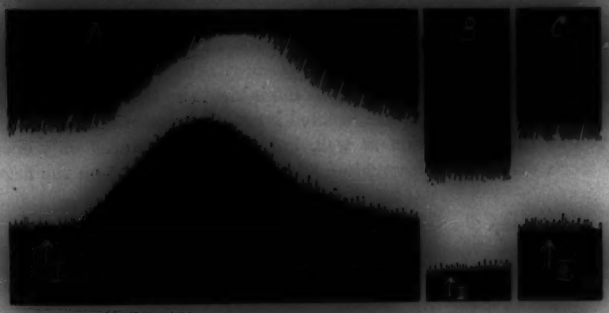

Fig. 11. Pituitrin.

A. Heart normal. Temp. 37·0° C. Heart rate = 148 per min. Outflow = 492 c.c. per min. B.P. = 130 mm. Hg. V.P. = 8·0 cm. H_2O. Heart's weight = 109 gm. At $\uparrow_I$. 0·1 c.c. Pituitrin was added. Transitory dilating effect. *B*. At $\uparrow_{II}$. 0·2 c.c. Pituitrin was added. Again normal. *C*. At $\uparrow_{III}$. 1·0 c.c. Pituitrin was added.

might be responsible. In order to investigate this possibility, the pressure was measured in the pulmonary system by inserting a cannula in one of the branches of the pulmonary artery and connecting it with a saline manometer. In Table IX, it can be seen that there occurred practically

TABLE IX.

Temp. 37·0° C. Heart rate = 158 per min. B.P. = 130 mm. Hg.
Heart's weight = 143·0 gm.

Time	System output c.c. per min.	Venous pressure cm. H_2O	Pulmonary pressure cm. H_2O	Addition of
1.25	500	10·5	14–16	
1.27	—	—	—	0·1 c.c. Pituitrin
1.31	500	13·5	15–17	Heart dilated
1.45	500	10·5	14–16	Heart volume again normal
1.48	—	—	—	1·0 c.c. Pituitrin
1.52	500	11	15–17	No effect

no change in the pulmonary pressure during the whole of the experiment, so that this could not be the cause of the heart dilatation. The concordance in the appearance and duration of the phenomena observed, both in the heart dilatation and diminished coronary flow, is so striking, that it makes it highly probable that in the case of Pituitrin they are in causal

relationship, the constriction of the coronary flow being the primary effect.

Quinidine. As it has been stated by v. Frey (15) and by Lewis and his co-workers (16) that Quinidine is more effective than Quinine, the alkaloid originally used by Wenckebach (17) in restoring the normal rhythm in cases of clinical fibrillation, and since the first was believed to be less toxic for the heart than the latter (Santesson (18), Valey and Waller (19)), it was substituted in therapeutics for the latter. According to Lewis and his pupils (20) Quinidine lowers the S.A. rate, reduces the rate of conduction in auricle and ventricle, depresses the A.V. conduction and lengthens the absolute refractory period of the auricular muscle. In view of these effects, it seemed desirable to test this alkaloid in the heart-lung preparation as well. A dose of 0·0066-0·01 gm. Quinidine sulphate was found to produce in addition to a bradycardia, a very well-marked heart dilatation (Fig. 12). The slowing of the heart beat would cause in itself

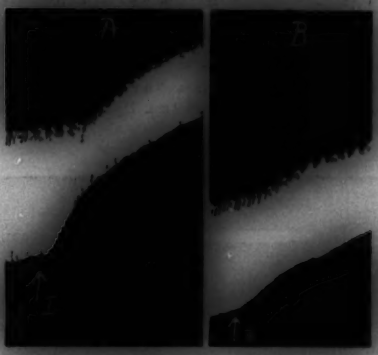

Fig. 12. Quinidine.

A = Heart normal. Temp. 37·0° C. Heart rate = 168 per min. Outflow = 480 c.c. per min. B.P. = 110 mm. Hg. V.P. = 7·0 cm. H_2O. Heart's weight = 83 gm. At †I. 0·0066 gm. Quinidine sulphate was added. Heart dilating. At †II. 0·0066 gm. Quinidine sulphate was added.

a dilatation by prolonging the diastolic filling of the heart. In order to investigate how far this is responsible for the heart dilatation the heart was artificially driven by means of rhythmic electrical stimuli during the whole of an experiment, before, during and after the administration of Quinidine, at a rate corresponding with the original rate of the heart.

Fig. 13. Quinidine.

Temp. 37·0° C. Heart artificially driven at a rate of 180 per min. Outflow = 429 c.c. per min. A.P. = 115 mm. Hg. V.P. = 6·0 cm. H_2O. Heart's weight = 78 gm. At † 0·01 gm. Quinidine sulphate was added.

Fig. 14. Quinidine.

Same exp. as Fig. 13 but later. At † 0·0166 gm. Quinidine sulphate was added. The ventricles do not follow the artificially driven auricles.

As can be seen in Figs. 13 and 14, though the complicating factor of bradycardia is excluded, the heart dilates after Quinidine. In Fig. 14 it can be observed, that after a second dose of Quinidine the ventricles are unable to follow the artificially driven auricles. Quinidine had no effect on the coronary circulation (Table X), unless given in a large dose, which caused, after a preliminary dilatation, constriction of the coronary vessels.

TABLE X.

Temp. 37·0° C. Heart rate = 144 per min. B.P. = 110 mm. Hg.
Heart's weight = 77·0 gm.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
4.40	—	29	48	
4.45	390	29	48	
5.52	—	30	50	
5.53	—	—	—	0·0066 gm. Quinidine sulph.
5.56	—	31	52	
5.59	390	31	52	
6.03	—	31	52	
6.05	—	31	52	
6.06	—	—	—	0·01 gm. Quinidine sulph.
6.07	—	36	60	Heart dilatation. Bradycardia
6.10	350	25	42	
6.13	—	24	40	
6.15	—	21	35	

DISCUSSION.

Reviewing the experiments, it can be said, that among the heart-tonics Digitalis and Caffeine really produce a tonic effect, because they enable the heart to expel the same amount of blood as before, with a smaller heart volume, the rhythm, venous inflow and arterial resistance being unchanged. The difference in their effect is only that after Digitalis the tonic effect develops slowly but lasts long, after Caffeine it is immediately apparent but has only a transitory character. Camphor and Strychnine, on the other hand, have no such effect; in the case of Camphor indeed, the effect is just the reverse, since it dilates the heart.

Among the other substances investigated, insulin produced a well-marked and lasting tonic effect, but this effect could be obtained only once, the second and further doses being ineffective. Pituitrin, on the other hand, dilated the heart, its effect appeared immediately after its administration, was of a transitory character, and usually could not be repeated. Quinidine, apart from bradycardia, produced also a heart dilatation, which was observed even after the exclusion of the change in

the heart rate. Sodium nitrite dilated the heart and Amyl nitrite had no effect at all on the heart muscle fibres.

As regards the mechanism of the tonic action, it may be considered as the direct opposite of that which occurs during the dilatation of the heart. As was shown by Starling and his pupils, if the work presented to the heart is suddenly increased, the heart fails for a time, and therefore gradually dilates. Immediately after the increase of the arterial resistance, the ventricle does not empty itself as completely as before, so that at the end of the systole the heart still contains blood, or, more blood than at the end of a previous beat. Since the ventricle is receiving a constant inflow from the venous reservoir, it is more distended in the next diastole than before. In the next systole the output is increased, but still not equal to the inflow, and this disparity becomes smaller at each beat for 3–10 beats, the output steadily increasing and the heart steadily dilating, until a new balance is attained, the output becoming again equal to the inflow, but the heart volume being increased. If the venous inflow is increased, the changes produced are somewhat similar, the output steadily increasing from its normal amount and the heart steadily dilating until it reaches again a condition in which the output becomes equal to the inflow with increased heart volume.

In our experiments, whether the work of the heart was augmented by increasing the arterial resistance or the venous inflow or both, when the tonic effect begins to be apparent, there is a transitory increase in the output and a gradual decrease in the heart volume until it arrives at a state in which the output is again equal to the inflow, but the heart volume is smaller. Starling and Visscher have shown that as the heart tires and dilates, in order to maintain the same work as before the total energy liberated increases with the increase in volume, and the mechanical efficiency of the heart accordingly diminishes. This rule was established by observations on hearts not treated with drugs, and it is a question whether it could be applied also to hearts under such influence. In the present paper this has not been investigated, oxygen consumption not being determined, so that the question cannot be answered definitely. It is hoped that these determinations may be made on a later occasion. If this rule is found to be valid even after the administration of a tonic, which is quite conceivable, this would mean that, as the heart volume becomes smaller, its functional capacity improves, its mechanical efficiency being increased.

In any case the decrease in volume obtained is the important direct therapeutic effect of the tonics upon the heart. The therapeutic effect on

the conductivity, which is according to Cushny (21) and Lewis (22) an effect partly on muscle and partly on the vagus, will not be discussed here. The conception which can still be found in various textbooks and papers, that one of the most important direct effects of Digitalis upon the heart is to increase the diastolic volume, is certainly incorrect. Just as a skeletal muscle has no power to lengthen itself actively after the contraction, but has to be lengthened by the application of some extending force, so, in the same way, the ventricles cannot dilate of themselves after systole, but must be distended by the inflow of blood; and the degree of this distension depends on the rate and pressure at which the blood enters the heart, the diastole of the ventricles being terminated by the next systole. In the heart-lung preparation the rate and pressure of the inflow and the rate of the beat being all constant, no increase in diastole could be observed after Digitalis; but in experiments performed in other ways and especially in experiments on the whole animal, this effect may be observed, because the slowing of the rhythm produced by Digitalis indirectly through the nervous system prolongs the time for the diastolic filling. Even after exclusion of the vagus action by atropine this increase of diastolic volume may be observed, because the general constriction of the vessels by Digitalis diminishes the total volume of the vascular system —thereby raises the pressure of the inflow. But these are secondary effects, and not the direct effect on the heart itself.

Further, from the results obtained, the conclusion must be drawn that with the exception of Pituitrin there exists no definite connection between the tonic effect of a drug on the heart muscle and its effect on the coronary vessels; since some had the same effects on the heart muscle but produced a quite different effect on the coronary flow, while others having the same effect on the coronary circulation produced a different effect on the heart muscle. Digitalis and Caffeine, for example, are heart-tonics and dilate the coronary vessels, but Insulin, which had the same effect on the heart muscle, had either no effect on the coronary flow or diminished it if given in larger doses (10 units), which still produced a tonic effect. On the other hand, camphor dilated the heart and increased the coronary flow, Pituitrin and Quinidine also produced a heart dilatation, but constricted the coronary vessels (in the case of the latter this was observed only after larger doses, small doses having no effect on the coronary flow). Even the two nitrites, though they had almost the same effect on the coronary circulation, behaved differently towards the heart muscle, Sodium nitrite dilating the heart while Amyl nitrite had no effect at all. In the case of Pituitrin the appearance and duration of the two effects

made it very probable that they are in connection, the constriction of the coronary vessels being a probable cause of the heart dilatation.

Concerning the effect on the coronary flow it has to be remembered that in the denervated heart-lung preparation the coronary vessels are deprived of the nervous control, which might cause a great difference in the effects of these drugs. It would be of interest to investigate the effects of these drugs upon the coronary vessels in the innervated heart-lung preparation also.

Summary.

1. Provided the heart rate, the venous inflow and the arterial resistance are maintained constant, the tonic action of drugs upon the heart is revealed in the heart-lung preparation by a diminution in the diastolic and systolic volume of the ventricles.

2. Judged by this criterion Digitalis, Caffeine and Insulin produce a tonic effect, enabling the heart to expel the same amount of blood with a smaller average heart volume. This effect is, after Digitalis, delayed but lasting, after Caffeine relatively rapid in onset and evanescent, and after Insulin it could be observed only once in each preparation.

3. Camphor, Sodium nitrite, Pituitrin and Quinidine produce dilatation of the heart.

4. Strychnine and Amyl nitrite have no effect on the tone of the heart.

5. Digitalis, Caffeine, Camphor and the Nitrites increase the coronary flow, Nitrites and Caffeine in a very high degree, Digitalis and Camphor only slightly.

6. Pituitrin, Quinidine and Insulin diminish the coronary flow, the two latter only in larger doses; in smaller doses these are without effect.

7. With the exception of Pituitrin there exists no obvious relationship between the tonic effect and that produced on the coronary flow.

I take this opportunity of recording my lasting debt of gratitude to the late Prof. Starling for help and inspiration in this work and in a wider field.

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THE CAUSATION OF THE ANŒSTROUS PERIOD.

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I. Introduction.

Under natural conditions the majority of mammals have definite anœstrous periods, during which the reproductive processes are in abeyance. Further it is known that under laboratory conditions of constant temperature and food supply, and also to a lesser extent under conditions of domestication, mammals will breed throughout a much greater seasonal range. In most laboratory rodents, for instance, reproduction will take place throughout the entire year, although a winter anœstrus is characteristic of the wild types. It would seem that the constant temperature under laboratory conditions might be a factor in the disappearance of anœstrus (1), and this suggests the obvious test of transferring the animals to winter atmospheric temperatures. Allen (2) reports negative results of experiments upon a small number of mice. While our experiments were in progress Lee (3) reported an investigation of a somewhat similar nature on rats, the animals being left in a natural atmospheric temperature varying from − 6° C. to − 3° C. At − 6° C. the length of cycle was much increased, but at − 3° C. no very marked increase (1·7 days) was found.

In the present series of experiments we first examined, during not less than four weeks, the œstrous cycles of 30 selected mice kept in a constant artificial temperature of 16·5° C. to 18·5° C. during January–February; the animals were then put for 4 weeks into an outside shed, no attempt being made to regulate the temperature, which ranged between a daily maximum and minimum of 5° C. to 10° C. As this produced no regular effect on the periodicity of œstrus, the animals were then put into cold store where the temperature ranged from − 1° C. to 1° C. After 45 days certain of the animals were mated, and were removed from cold store if and when pregnancy ensued.

The detection of œstrus, copulation, etc. was by the methods previously described (4). During the whole of the experiments an unlimited food supply was allowed.

II. Experimental Results.

Control experiments. One hundred and eighty-two œstrous cycles were observed before treatment. The length of the dicestrous intervals in these cycles is shown in the following frequency distribution.

Table I. Length of dicestrous intervals at laboratory temperatures (16·5 to 18·5° C.) before treatment.

Length of interval in days	No. observed	Total days occupied
2	52	104
3	53	159
4	31	124
5	17	85
6	11	66
7	2	14
8	2	16
9	5	45
10	3	30
12	4	48
16	1	16
25	1	25
Total	182	732

The mean length of dicestrus before treatment was thus 4·02 days, and the distribution gives $\sigma = 2{\cdot}81$ and a p.e. of $\pm$ 0·14.

Length of dicestrus at outdoor temperatures. Under the outside shed conditions 86 dicestrous intervals were observed[1]. The average length of these intervals was $5{\cdot}65 \pm 0{\cdot}30$. This is longer by $1{\cdot}63 \pm 0{\cdot}33$ days than that found for the pre-treatment cycles, but as shown by Table II, the most obvious result of putting the animals under outdoor conditions was to increase the variability of the length of dicestrus.

The variability in this series ($\sigma = 4{\cdot}16$) is definitely greater than that found in the pre-treatment cycles ($\sigma = 2{\cdot}81$).

Length of dicestrus in artificially low temperatures. Whilst the animals were kept in cold store, 201 dicestrous intervals were observed. The mean length was $4{\cdot}97 \pm 0{\cdot}171$ days, which is only 0·95 days longer than before treatment, and which suggests that no effect whatever had been produced by the low environmental temperature. Examination of the individual

[1] As an arbitrary method of assessment the dicestrous interval was placed to one period or the other according to the incidence of its mid-point.

TABLE II. Frequency distribution of length of dioestrus under outside conditions (5° C. to 10° C.).

Length of dioestrus in days	No. of intervals	Total days
2	10	20
3	21	63
4	13	52
5	19	95
6	2	12
7	4	28
8	2	16
9	2	18
10	3	30
11	2	22
12	2	24
13	1	13
14	1	14
18	1	18
19	1	19
20	1	20
22	1	22
Total	86	486

œstrous histories, however, showed that in nearly all cases the first dioestrus in cold store was much prolonged. The dioestrous intervals occurring in cold store were therefore grouped according to the incidence of their mid-point. The results were as follows:

TABLE III. Length of cycle according to time in cold store.

Weeks in cold store	Length of dioestrus																	Total intervals	Mean length
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1	1	1	1	1	–	–	1	4	2	3	–	3	–	2	4	1	1	25	11·16 ± 0·59
2	2	4	7	3	1	–	–	–	–	–	–	–	–	–	–	–	–	17	
3	3	3	2	7	2	–	3	1	2	1	3	–	–	–	–	–	–	27	
4	14	8	5	–	1	1	–	1	–	–	–	–	–	–	–	–	–	30	
5	3	6	13	2	1	–	–	–	2	–	–	1	–	–	–	–	–	28	4·09 ± 0·12
6	3	14	9	1	–	1	1	–	–	–	–	–	–	1	–	–	–	30	
7	4	13	5	–	1	–	–	–	–	1	–	–	–	–	–	–	–	24	
8	3	11	5	1	–	–	–	—	–	–	–	–	–	–	–	–	–	20	
Total	33	60	47	15	6	2	5	6	6	5	3	4	0	3	4	1	1	201	4·97 ± 0·171

The initial increase in length of dioestrus is obvious from this table, and comparison of the initial and subsequent length of dioestrus in the cold store with the pre-treatment length gives the following results:

Length of dioestrus during	Difference from pre-treatment length	P.e. of difference	Difference/p.e.
1st week in cold store	+7·14 days	±0·61	11·7
2nd to 8th weeks in cold store	+0·07 days	±0·18	0·389

The following conclusions may be drawn from these results:

(*a*) The first effect of much reduced temperature is to lengthen the dioestrous interval.

(*b*) Subsequently, however, the length of cycle becomes indistinguishable from the normal, and no sign of anœstrus is found.

There is, therefore, no simulation of the prolonged winter anœstrus of wild rodents; the subsequent return of the cycle to normal suggests that the experimental animals were able to adjust themselves to the low temperature, and that in the absence of such adjustment anœstrus might have resulted.

The food consumption was, however, much higher at the lower temperature, and it was perhaps this which made the adjustment possible. With the wild mammal no such liberal food supply can be available during the winter, and it has been shown experimentally (5, 6) that an insufficient food supply leads even at normal temperatures to increase in the length of dioestrus. It would appear quite possible that the anœs-

No. of animal	No. of infertile copulations	No. of œstrous periods without copulation	Size of litter	Sex of young	
				Males	Females
CC 3	0	0	7	4	3
CC 5	0	0	Definitely pregnant at 13 days but apparently re-absorbed		
CC 9	0	0	6 in uterus when killed	–	–
CC 10	0	1	8	3	5
CC 14	0	0	6	4	2
CC 15	0	1	3 (some eaten)	3	–
CC 16	0	0	5	4	1
CC 18	0	0	Pregnant when killed	–	–
CC 19	0	4	–	–	–
CC 22	0	2	4	2	2
CC 23	0	1	9	4	5
CC 25	1	1	–	–	–
CC 27	0	0	8	5	3
CC 28	0	0	Aborted	–	–
CC 29	2	0	–	–	–
CC 32	2	0	–	–	–
CC 34	0	0	2	2	0
CC 38	0	0	Definitely pregnant at 12 days but re-absorbed		
CC 39	0	1	Definitely pregnant at 11 days but re-absorbed		
CC 40	0	0	Died in parturition	–	–
CC 42	1	1	–	–	–
CC 44	3	0	–	–	–
CC 45	0	0	7	3	4
CC 46	0	0	5	2	3
CC 47	0	0	Definitely pregnant at 15 days but re-absorbed		

trous period of the wild rodent results from decrease in both temperature and food supply.

Breeding performance at − 1 to + 1° C. The above table sums up the performance of the 25 animals which were mated in the cold store with normal males.

In these mated animals 12 œstrous periods unattended by copulation were observed, but only one animal failed to copulate at any time. Infertile copulations (nine in all) were observed in five mice. Nineteen of the 25 thus became pregnant. The excessive number of cases (five) where the pregnancy terminated prematurely by re-absorption or abortion of all the fœtuses was probably due to the sudden change of environment on being taken from the cold store.

III. Conclusions and Summary.

(1) A small decrease in environmental temperature has no marked effect on the œstrous cycle in mice except that the variability in length is somewhat increased.

(2) A considerable decrease, however, from the ordinary temperature of 18° C. to a mean of about 0° C. causes an initial increase in the length of diœstrus ($4{\cdot}02 \pm 0{\cdot}14$ to $11{\cdot}16 \pm 0{\cdot}59$). Very shortly, however, the normal periodicity of œstrus is restored, and even prolonged exposure to the low temperature produces no further effects on the reproductive processes. When mated the animals are normally fertile.

(3) The prolonged winter anœstrus of wild rodents is probably to be attributed to a diminished food supply under conditions of temperature which, for the maintenance of normal function, would require an increase in food.

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THE EFFECT OF TEMPERATURE ON THE ACIDITY OF THE SKIN.

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THE studies of Stadie and Martin(1), Austin and Cullen(2), and Stadie, Austin and Robinson(3) have demonstrated that blood *in vitro* becomes more acid when it is warmed, unless CO_2 can escape. It is consequently important to decide whether the same changes occur *in vivo*, and if so, whether the vascular dilatation produced by heat is in any way dependent on such changes. Rous and his co-workers(4) have described the injection of large amounts of indicator dyes into mice and other animals in order to determine approximately the acidity of the tissues. It has been found that his method may be adapted to cats and used for the estimation of changes in acidity in the skin.

Method. White cats of 3 to 3½ kilos weight have been used; they were decerebrated under a brief chloroform-ether anæsthesia by the Sherrington guillotine method(5), care being taken to divide the brain stem between the posterior colliculi and the anterior margin of the pons, so as to have a quiescent preparation. The two fore limbs were then shaved between the elbow and wrist joints, and the hyperæmic response to various conditions was estimated by comparing the colour of the skin of the two limbs. After these observations had been made, about 30 c.c. of a 2 p.c. solution (approximately neutral) of phenolsulphonephthalein was injected intravenously into a vein of the hind limb. After an injection the cats had a purplish pink colour, which was readily visible through the hair and which was intense on the shaved areas. The colour developed within three-quarters of a minute and reached its maximum in less than two minutes. The dye was rapidly excreted by the kidneys and the colour was fading rapidly two to four hours later. The colour of the skin, as a whole, was definitely towards the alkaline side of the indicator range, but that of the ears, occasionally, was quite yellow, since both common carotid arteries had been tied. Afterwards the ears usually resumed a more alkaline colour. The shaved legs, which were cold as a result of exposure and evaporation of water, also had a

definitely purplish tint (similar to that of a standard solution of the dye of a concentration of 0·001 p.c. with a *p*H of 7·6 at 20° C. and contained in a test tube of 13·3 mm. internal diameter). Where the skin had been abraded in the process of shaving, the colour was of a deeper purple tint, either through removal of cuticle, which would otherwise dull the colour, or, more probably, through an actual increase of alkalinity due to loss of CO_2 from the surface. The wounds due to the operative procedures left uncovered by skin assumed a very alkaline purplish colour, suggesting a *p*H of at least 7·8 or more. The pads of the feet, where unpigmented, had a less purplish tint than the skin of the shaved areas.

Measurement of subdermal temperatures in the wet shaved forelegs in one animal made by loop-thermocouples (6) indicated temperatures of 23° to 27° C. The rectal temperature of the cats has been maintained as constant as possible and has usually remained between the limits of 36° and 39°.

For purposes of determining the effect of temperature, both before and after injection, the cats were suspended with their heads raised and the two fore limbs immersed in water in rectangular glass museum jars; the water was not stirred except immediately after the initial insertion. Behind the glass jar an opal glass plate was adjusted, so as to afford a constant background. One of the limbs was used as a control, and colour differences between the two limbs were noted; an attempt was made to express any difference in tint according to a scale of colour variation with *p*H at 20° C. in standard tubes of the type already mentioned. Such colours are mentioned so as to give an approximate idea of the actual tint; it was not possible to estimate the exact *p*H level, since a colour match was prevented by the opaqueness of the tissues, by irregular errors introduced by varying amounts and degrees of saturation of hæmoglobin, as well as by unmeasurable salt and protein errors. The colour changes were, however, often definite, and must have indicated the direction of the changes in acidity and approximately their magnitude, in spite of the above-mentioned errors.

The effect of circulatory arrest on the acidity of the skin has been tested. "Riva Rocci bags" made of rubber finger stalls were tied on to glass tubes and bandaged lightly over the humerus and elbow joint with a 1-inch bandage. This system was connected to a mercury manometer. The pressure could be raised to over 200 mm. in less than two seconds. Stasis was maintained for varying periods up to 20 min. The legs were employed alternately. If stasis had been applied for 5 min., an interval of at least 12 min. was left before that leg was used as a control, and at

least 25 min. before it was used for any further experimentation. If stasis had been applied for 20 min. these periods were extended to 15 and 50 min. In addition, the colour of the control leg was determined not to be altering appreciably before any further experiments were made.

Two abbreviated protocols are appended and will serve to illustrate the character of the experiments, the time intervals allowed and the type of change noted.

Protocol 1. Cat, male, 3·2 kg. 9.35 to 9.50 decerebrated. 10.19 rectal temperature 39·2°. Slight hæmorrhage from brain stem subsiding. 10.25 opisthotonos which disappeared later. 10.30 shaved and "Riva Rocci" applied. 10.59 with both forelegs in air, an arrest of circulation was maintained by 150–180 mm. Hg. for 5 min. Thirty seconds after release the shaved areas and the paws became hyperæmic; at 1 min. 30 sec. the hyperæmia persisted without perceptible change, and at 2 min. 45 sec. a trace of hyperæmia was present.

Table I details a series of consecutive arrests of circulation for varying periods, with the forelegs immersed in water at an intermediate temperature. Time in minutes (′) and seconds (″).

12.50 rectal temperature 39·0°. 1.38 30 c.c. 2 p.c. solution of phenol red injected. The colour of the skin matched that of a standard tube of *p*H 7·6. 1.43 rectal temperature 39·7°.

Table II details a series of consecutive arrests of circulation for varying periods, with the forelegs fully immersed in water.

Protocol 2. Cat, female, 3·1 kg. 10.15 to 10.30 decerebrated. 11.17 rectal temperature 38·0°. 11.20 shaved. "Riva Rocci" apparatus applied to both forelegs above the elbow, but on the right the bandage covered part of the elbow. Both legs at this time showed slight congestion.

Table III details a series of consecutive circulatory arrests of the same duration but with the temperature of the bath varied.

1.12 rectal temperature 38·0°. 2.21 rectal temperature 37·0°. 2.45 30 c.c. 2 p.c. solution of phenol red injected. 2.49 both forelegs into bath of 30·5°.

Table IV details a second series of consecutive arrests circulatory for comparison with the previous series.

4.46 both forelegs were partially immersed in water of 40·5°. The acidity of the skin of immersed portion definitely greater than that of the skin which was not in the bath. 4.49 the forelegs were completely immersed, the right in a bath at 36°, the left in one at 18·5°. The line of previous submergence was recognisable on both forelegs by a greater intensity of colour, which within 1 min. had disappeared on the right foreleg (36°) though persistent on the left (18·5°). This increased colour in the left leg disappeared after 6 min. to 7 min. 5.12 a $\frac{1}{1000}$ solution of ergamine acid phosphate (histamine) was introduced into the skin and subcutaneous region of the left leg by the method of Lewis and Grant(8). The lower part of the right leg was scalded with boiling water. No change of colour was noted in either limb.

5.20 application to left foreleg of histamine repeated, employing $\frac{1}{1000}$ solution, and again the same area of the right foreleg was scalded. Circulation arrested under pressure of 200 mm. Hg. 5.24 arrest terminated; the right foreleg definitely acid, most marked where previously scalded. 5.25 the centres of the histamine-affected areas appeared to have a more yellow tint than the rest of the leg. 5.27 circulation arrested in bath 31·5°. 5.31 arrest terminated. Contrast between inflamed and normal areas unchanged during and after arrest.

TABLE I.

Time arrest initiated		Duration of arrest	Pressure within compressing system. Mm. Hg	Leg used in experiment	Temp. of bath °C.	Changes during period of stasis	Period of release				
Hr.	Min.						Appearance of flush on release	Maximum flush	Fading begins	Fading advanced	Colour normal
11	20	1′	160	Left	34·5	—	10–11″	45″	45–75″	—	75–80″
11	36	5′	200	Right	36·0	Skin first pale then cyanotic	10–12″	37–39″	60″	—	90″?
11	58	10′	180	Left	35·0	Cyanosis perceptible on shaved skin 30″ and on dorsal area of toes 4′ 30″ after arrest	9–10″	20–30″	70–120″	4′ 45″–5′ 30″	6′ 30″
12	25	10′	200	Right	35·0	Cyanosis perceptible 73″	9–10″	30–80″	70–120″	4′ 30″–6′ 30″	8′ 30″

TABLE II.

Time arrest initiated		Duration of arrest	Pressure within compressing system. Mm. Hg	Leg used in experiment	Temp. of bath °C.	Changes during period of stasis		Period of release				Remarks
Hr.	Min.					Acidity detectable	Ultimate colour obtained. Maximum	Increase in acid colour	Reversal towards alkalinity	Trace of acidity	Return to normal	
1	54	30″	200	Left	34·5	—	—	30″ ?	—	—	—	After this test right leg approximate pH 7·6, left pH 7·5
1	57	1′	200	Right	34·5	—	—	10–20″	—	60–95″	—	
2	05	5′	200	Left	34·5	16–20″	pH 7·0	6–25″	60–80″	4′ 30″	5′	
2	25	10′	200	Right	35·5	20–30″	pH 6·9	5″	60″	4′ 30″	5′	
2	53	15′	200	Left	36·0	15–45″	pH 6·8	immediate	11–45″	6′ 30″	11–12′	
3	35	The right leg was placed in a bath of 12·5° with the circulation intact; when equilibrium was established the colour present corresponded to a standard of pH 7·5. Similarly the left leg in a bath of 40·5° had a colour on this basis of pH 7·2. Arrest of circulation gave the following results.										
3	52	10′	200	Right	12·5	Possibly slight change to acid side		Any further change not perceptible				
3	52	10′	200	Left	40·5	30″	Lower than pH 6·8	—	18–20″	2′ 30″	3′ 30″–4′ 30″	

TABLE III.

Time arrest initiated			Pressure within compressing system. Mm. Hg	Leg used in experiment	Temp. of bath °C.	Changes during period of stasis	Period of release				
Hr.	Min.	Duration of arrest					Appearance of flush on release	Maximum flush	Fading begins	Fading advanced	Colour normal
11	35	10′	200	Left	40	Marked cyanosis	20″	30″	50″	18′	—
12	10	10′	200	Right	41	Very marked cyanosis	30″	120″	2′ 30″	9′	11′ 30″
12	51	10′	200	Left	30	Cyanosis developed slowly 30–60″. Definite cyanosis 1′ 45″	45″	60″	1′ 45″	8′	10′ 30″
1	16	10′	200	Right	30	Cyanosis 45″. Marked cyanosis 60–90″	45″	75″	2′ 30″	5′	5′ 30″?

TABLE IV.

Time arrest initiated			Pressure within compressing system. Mm. Hg	Leg used in experiment	Temp. of bath °C.	Changes during period of stasis		Period of Release				
Hr.	Min.	Duration of arrest				Acidity detectable	Ultimate colour obtained. Maximum	Increase in acid colour	Reversal towards alkalinity	Trace of acidity	Return to normal	Remarks
2	51	10′	200	Left	30·5	90″	*p*H 7·5–7·4	60″	90–105″	4′ 45″–5′	7′ 30″	Control (right) leg *p*H 7·6
3	15	10′	200	Right	29·5	—	*p*H 7·4	45″	105″	7–8′	8′–8′ 30″	Control (left) leg a little on the acid side of *p*H 7·6
3	51	10′	200	Left	40·5	60″	*p*H 7·0	30″	45–60″	10′	10′ 30″?	Control (right) *p*H 7·4
4	18	10′	200	Right	41·0	45–60″	*p*H 7·0	45″	45–60″	6′	5′ 30″–6′ 30″	Control (left) *p*H 7·4

Results.

Acidity in the skin as affected by temperature with the circulation intact. Cooling of one limb, while the temperature of the other was maintained at an intermediate level (31° to 35°, usually 32° to 33°), produced a tint generally definitely more purple than that of the control. Warming of one limb to a higher temperature decreased the visible colour and often gave it a slightly more orange tint. Occasionally the two limbs could be put into water at very varying temperatures without any definite colour difference. As a rule, however, a colour difference could be detected if the temperatures of the water in which the limbs were immersed differed by more than 8 to 12° C. The warmer limb always appeared the more acid in spite of errors from any vascular dilatation which might be expected to minimise any colour difference. The subdermal temperatures were close to those of the water; in one experiment where subdermal temperatures in the shaved areas were recorded by thermocouples, while the limbs were immersed in water baths which were stirred, the temperatures recorded differed from those of the baths by less than 1° C.

The maximal difference in tint recorded is given in Protocol 1. The right leg at 12·5° had a colour similar to that of a tube of *p*H of 7·5, and the left in water at 40·5° was similar to that of a tube with *p*H 7·2. But the dissociation of the dye is affected by temperature, so that if these values be corrected for temperature, according to the data of Hastings and Sendroy(7) (which demonstrate that a correction of − 0·007 *p*H per 1° increase of temperature should be applied), the actual difference in *p*H would be more nearly 0·5, and the *p*H of the warmed limb would be probably in the range of 7·1. Errors already considered make the absolute value 7·1 very uncertain and of little importance, but the difference of 0·5 between the two limbs should be approximately true. Very large differences may, therefore, be seen *in vivo*. The smallest difference observed in experiments of this type was as follows: the right leg was immersed in water at 33°, the left at 15°. The right was considered to have the more purple colour, but the difference was indefinite and uncertain. If, however, the colours were actually identical and temperature corrections were applied, the *p*H of the cooler limb would be at least 0·1 greater than that of the warmer.

In another experiment, one leg was warmed considerably and the other cooled until a definite difference in tint was noted. Both legs were then removed from the baths and were exposed wet to the room air; subdermal temperatures were determined by thermocouples and recorded

photographically. No difference in colour could be detected, unless the temperature difference between the two legs exceeded 12° to 15°.

It may, therefore, be concluded that, in spite of the well recognised changes in the circulation rate, on exposure to cold the acidity of the skin is reduced, while on warming it is increased.

Acidity developed during arrest of the circulation at intermediate temperatures. If the legs were immersed side by side in water at a temperature of 31° to 35° (usually 32° to 33°) and the circulation was arrested in one limb, the colour gradually altered towards a yellowish tint in spite of the simultaneous development of cyanosis. Protocol 1 gives an example of this in an experiment where the acidity developed was somewhat greater than that usually observed. The acidity reached during stasis, and the time required on the release of the circulation to return to the initial colour varied with the duration of stasis; the time values recorded were not very different from those noted for reactive hyperæmia following similar occlusions before the injection of the dye. Though no exact parallelism in duration was to be observed, both phenomena could often be distinguished for 5 to 10 min. The absence of any exact parallelism is not significant, since the condition of the animal was not necessarily the same in the two series of experiments, and in bothcases the colour differences were difficult to time exactly. The reactive hyperæmia was less conspicuous in the cats than in experiments on man. Both the development and disappearance of the acid colour in the skin showed a somewhat patchy distribution; the skin was everywhere acid, but the degree of acidity apparently differed in different areas.

The effect of temperature on the acidity developed during circulatory arrest. If one leg was immersed in cold water and the other in warm, and if after some minutes the circulation was arrested in both simultaneously, the warmed leg rapidly developed an increased acidity, while the cooled leg showed no appreciable change in colour (see Protocol 1). In one animal a series of circulatory arrests were made at two temperatures both before and after injection of the dye, and the data are presented in Protocol 2. An intensification of the reactive hyperæmia by warmth was noted (confirming the earlier observations of Lewis and Grant(8) on man), and at the higher temperature on this occasion it also lasted longer after release. The acidity developed was much greater at the higher temperature, and its duration on release was also greater, though the time differences were less marked than in the series with reactive hyperæmia. It may be noted that the acidity developed in all the experiments was greater the higher the temperature of the bath, but that sometimes the period of recovery

on release of the circulation was not increased but decreased by the warmth (see Protocol 1, where the period appeared to be slightly longer in a bath at 35° than in one at 40·5°).

Experiments to test any possible relationship of acidity to circulatory changes. Since it seemed possible that the hyperæmia produced by heat was secondary to the acidity developed, some of the ingenious experiments of Lewis and Love(9) have been repeated on these preparations. These workers demonstrated that the hyperæmia produced in the lower part of one limb by immersion in water at 41° to 42°, with subsequent total immersion of the limb in water at 20° or 32°, persisted longer at the lower temperature. It has been found possible to confirm their observations on decerebrate cats, and an experiment of this type on an injected animal is detailed in Protocol 2. While the persistent hyperæmia was indicated by an intensification of the colour, no increased acidity was demonstrable in the area affected. If any acidity persisted, the colour change must have been so slight as to be masked by the hyperæmia. Lewis and Love also demonstrated that the hyperæmia following stasis in water at 20° is of longer duration in an area which had been exposed immediately before the production of stasis to a temperature of 40° to 42°. These experimental results can also be confirmed in decerebrate cats, but again no very definite increase in acidity was demonstrable in such cases.

The two forelegs of an injected decerebrate cat were immersed in water at 32°, when the colour of both legs was about equivalent to a standard tube of *p*H 7·6. They were then half immersed in a bath at 41·5°, in which the immersed portion of both legs developed a colour equivalent to a standard tube of *p*H 7·4, while above the water the colour was more purple than a 7·6 standard. After 9 min. the circulation of the left leg was arrested. Immediately both legs were totally immersed in water at 28°. Stasis was maintained for 9½ min. In the right leg with circulation intact no definite line of demarcation due to the previous heating was visible, although the colour of the lower part of the right foreleg was somewhat more intense. After 2½ min. this difference in intensity of colour had diminished until its existence was questionable. In the left leg the colour gradually became less red than on the right, but no difference in either tint or colour intensity between the upper and lower part of the leg was detectable. At the end of 9 min. the colours were, right 7·6 and the left slightly on the acid side of 7·4. On the release of the circulation the upper part of the left leg was more yellow than the lower; later the lower part became more intensely coloured than the upper part and the line of previous heating was well defined. For a brief period at this time the colour below this line appeared the more yellow. On repeating the experiment with an arrest of circulation in the other foreleg for 5 min., the changes were similar but the lower part of the limb, which had been previously heated, appeared to develop a slightly greater acidity than the upper.

The evidence is indefinite, whether the greater acidity in the tissues developed as the result of exposure to temperatures of 41° or more can persist even after the leg has been removed to water at a lower temperature. It does not seem likely that changes in acidity so difficult to detect

can entirely explain the marked hyperæmia which can be demonstrated under these circumstances. In one experiment a heat hyperæmia appeared to be induced and to persist longer with later immersion at a lower temperature, when the temperature of the first bath was 40·2° on insertion of the legs and 39·4° on withdrawal 5 min. later. The rectal temperature was 38·7° at this time, and it is somewhat difficult to conceive that a bath at this temperature could produce an inflammatory reaction, which could account for the changes.

Inflammation. Since it was possible that the reaction to high temperatures was really an inflammatory response, some experiments were made on other types of inflammation. Some of these experiments are detailed in Protocol 2. If a number of pin-pricks were made at distances of 1 to 2 mm. from one another, and the leg was immersed in warm water, no difference in colour between the affected and control areas was detectable, either in tint or depth, except where the pin-hole was large, when the centre might be recognisably alkaline. If a similar set of punctures was made through a $\frac{1}{300}$ solution of ergamine phosphate, a slight yellowish colour was sometimes noticed. If a stronger solution ($\frac{1}{150}$) was used a yellow colour could be distinctly seen, which lasted only a short while. A $\frac{1}{100}$ solution resulted in a more definite yellow colour, also of brief duration. The acidity of the salt would account for it, and it is probably not significant. No difference in depth of colour could be detected around these histamine punctures.

If inflammation was produced by hot glass or boiling water, a marked hyperæmia developed, and usually in the centre of this area a small region of a somewhat yellowish tint could be distinguished.

Experiments on human subjects. Attempts have been made on two human subjects to repeat some of these observations. A solution of the dye (0·6 p.c.) was injected into both forearms, either subcuticularly or subdermally. For the former a small amount of dye was used sufficient to infiltrate an area of 7 to 10 mm. diameter to a rose-purple colour, and several such areas were infiltrated in each arm. For the subdermal injections, $\frac{1}{2}$ c.c. of the solution was injected. Immediately after subdermal injection no colour was detectable, but within a few minutes the skin assumed a rose purple.

The effect of temperature on the acidity with the circulation intact was similar to that observed in cats but much less marked. In both subjects immersion in baths differing by 25° gave a difference in tint probably corresponding to less than 0·1 *p*H of the standard tubes. This difference in acidity if corrected for temperature would be probably about 0·2 *p*H, the arm in the warm bath was the more acid.

When the circulation was arrested for 20 min. in baths at 42° to 39° a definite acidity was developed in both subjects, but if expressed in the same terms as those used for the cats, the change in acidity probably was not more than 0·2 *p*H even at this high temperature. On release of the circulation a difference in tint suggesting acidity could be noted for 4 to 7 min., though the hyperæmia was intense for a much longer period. In an experiment with a water bath at 22° the development of acidity during a stasis of 20 min. was quite indefinite below the water line, but above, where the skin was warmer, a slight but definite difference could be detected. Hyperæmia long outlasted any detectable acid colour.

Discussion.

The dye when injected into the blood stream of a cat probably passed rapidly into the tissue fluids and could be recognised soon after injection in the mesenteric lymphatics[1]. The estimation of acidity was probably influenced by both the acidity of the blood and the tissues. The circulatory changes observable in decerebrate cats after such procedures as temporary arrest of the circulation resembled those observed in man more closely than do those recorded for anæsthetised animals by Goldblatt(10).

The data obtained by Stadie and Martin and by Austin and his co-workers(1, 2, 3) demonstrate that human blood probably has a decrease in *p*H of about 0·02 per 1° C. rise in temperature, if the total content of CO_2 remains unchanged. The maximum change with temperature estimated in cats in our experiments, when the circulation has been intact, has been of about this order; usually the alteration has been less. Possibly the increased circulation rate in the skin with diminished venosity of the blood is a compensatory mechanism. The data of Schierbeck(11) also suggest that loss of CO_2 from the skin is not a negligible factor and is greater the higher the temperature.

But in addition to the effect of temperature on the blood, the metabolism of the tissues is likely to be altered. A diminished oxygen consumption at low temperature has been demonstrated by Goldschmidt and Light(12). The experiments involving stasis here reported indicate quite definitely that at low temperatures, not only is oxygen consumption diminished, but also acid production is reduced so as not to be measurable. On the other hand, at high temperatures acid production is extremely rapid and the effect parallels that of temperature on the production of cyanosis with stasis. Any compensating factors, which

[1] We are indebted to Dr R. J. Brocklehurst for this observation as well as for assistance in some of the experiments.

make the acid changes *in vivo* less than those observed with blood *in vitro*, must neutralise not only the changes in the physical constants in the blood (mainly an alteration in the acid constants of the blood proteins) but also the variations in acid production of the tissue. Any compensation is incomplete; the tissues of the skin are more alkaline when cooled and not more acid, though on account of the changes in circulation rate an increased acidity might have been anticipated. The end result would appear to be a balance between increases in the acidity of the blood and acid production of the tissues with a rise in temperature and a partial decrease in blood acidity through an alteration in the blood flow and loss of CO_2. Alterations of any of these factors would account for the considerable individual variation seen in different animals. The question arises whether the circulatory changes are dependent upon the changes in acidity. A vascular dilatation to acidity is well recognised (Leake, *et al* (13)) and it is not unlikely that the vascular dilatation of reactive hyperæmia is due, either in part or entirely, to acid metabolites produced during the arrest. In favour of such an hypothesis is the fact that conditions which vary the intensity or duration of the reactive hyperæmia also vary the intensity and duration of the acidity. The fact that a high temperature may either increase or decrease the duration of measurable acidity after an arrest of circulation, may depend on an irregular balance between the greater acid production and the increased rate of blood flow tending to remove the acid. It is noteworthy that Lewis and Grant(8) reported no constant effect of temperature on the duration of reactive hyperæmia following occlusion; the effect of temperature on the duration of acidity is also indefinite and irregular. The same workers demonstrated plethysmographically a much greater dilatation at the higher temperatures; the acidity developed is also greater at such temperatures.

The variation in blood flow with temperature, when the circulation is intact, could be explained also on a simple variation in alkalinity, if the response to temperatures above 40° to 42° be excluded. At these higher temperatures a hyperæmia is induced which is preserved after immersion of the part in cold water without any definitely measurable acidity; this may best be ascribed to some other factor such as the H-substance of Lewis(14). In fact, though the data reported suggest the possibility of acid being mainly concerned, in no case has the action of some other substance been excluded.

A few experiments on inflammatory reactions are reported; some evidence was obtained of acid production as the result of injury, but the acidity observed does not seem adequate to account for the marked circulatory changes. The acidity noted could be explained as the result

of the phosphoric acid of the histamine salt, or in the case of the scalds, as the consequence of a small local area of stasis. Any fresh open wound has apparently a very alkaline reaction, but it is doubtful whether an inflammatory reaction is absent.

Observations made on man indicated changes in the same direction but of much smaller magnitude. It is uncertain how far the differences were due to the limitations of the technic. It is possible that the acid production occurs to a large extent in the muscles and that the effect on the skin is less, owing to the greater thickness of the intervening tissues.

It is suggested that there may be two chemical mechanisms through which the local circulation may be varied when the temperature is altered; one occurring at extreme temperatures when the tissues are injured and possibly due to liberation of the H-substance of Lewis, and a second utilised at all temperatures and dependent on acid-base adjustments.

Conclusions.

(1) Acidity changes can be demonstrated in the skin of a decerebrate cat injected intravenously with phenolsulphonephthalein.

(2) The acidity varies with the temperature; a leg immersed in a warm bath shows a greater acidity than one immersed in a cold bath.

(3) Acid production during an arrest of the circulation is considerable, and is greater the higher the temperature. After a long arrest, the acidity may not disappear for 5 or 10 min. following release of the circulation.

(4) Hyperæmia produced by immersion in water at 40° to 42° and persisting when the leg is subsequently immersed in water at a lower temperature does not appear to be associated with measurable acidity.

(5) The possible relation of local vascular changes to temperature and of reactive hyperæmia to changes in acidity is discussed.

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PHOTOGRAPHIC METHODS OF ESTIMATING THE PERCENTAGE SATURATION OF HÆMOGLOBIN WITH VARIOUS GASES. I. The ratio of oxyhæmoglobin to carboxyhæmoglobin.

By H. HARTRIDGE and F. J. W. ROUGHTON.

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Introduction.

The importance of being able to estimate with accuracy the ratio of oxyhæmoglobin to carboxyhæmoglobin lies in the fact that there are several purposes to which such a method can be applied, amongst which are:

(1) The detection of mild degrees of carbon monoxide poisoning due to leakage either of coal gas or of the products of incomplete combustion into the air which is being breathed.

(2) Experimental work on the kinetics of hæmoglobin and one or both of these gases, either on blood corpuscles or on solutions of hæmoglobin.

(3) The determination of the total amount of hæmoglobin in the circulating blood by the method of Douglas and Haldane[1]

The carmine method of Douglas and Haldane by which they estimated the degree to which carbon monoxide gas had replaced oxygen from its combination with hæmoglobin had the disadvantage that only a single determination could be made on one sample of solution.

The reversion spectroscope of Hartridge[2, 3] did not suffer from this disadvantage since by its means a large number of determinations could be made on one sample.

Both these methods suffer however from the disadvantage that neither of them obtains from the sample a permanent record which can subsequently be subjected to remeasurement and verification, and both methods are liable to subjective errors.

It was largely with the object of obtaining permanent records that the photographic methods to be described in this paper were investigated.

The results show that the photographic method would appear to

have as wide a scope as the reversion spectroscope and to have two advantages additional to those already referred to:

(*a*) The accuracy is in general higher than that attainable, even under the best conditions, by the reversion spectroscope. Especially is this the case when the solution contains a high p.c. COHb.

(*b*) The time required for making the record can (by increasing the intensity of the source of light used for photographing the solution) be reduced to a few seconds, and thus economise greatly the amount of fluid required in cases where observations have to be made on moving fluids (*e.g.* for purposes of kinetic studies).

The Photographic Method.

Inspection of the spectrophotometric data obtained by Hartridge (2) shows (*a*) that the movement of the α band, which occurs when oxygen gas is replaced by carbon monoxide gas from its combination with hæmoglobin, is greater than the movements of the β bands or of the trough between the two bands, and (*b*) that the edges of the α band are sharper than those of the β band. For these reasons our photographic records were limited to the spectrum occupied by the α band.

Since the α band lies in the yellow-green part of the spectrum to which ordinary non-colour sensitive photographic plates are practically insensitive an orthochromatic or panchromatic plate had to be found that would be suitable for our purpose. After testing several different makes and varieties it was found that Ilford Chromatic plates (half-plate size) were suitable.

For reasons which will be discussed later it was desirable that the α band should appear on the negative as a light band bounded on either side by areas of equal blackness, *i.e.* should be as symmetrical as possible. Reference to the absorption curves of O_2Hb and COHb shows that in both cases the area of the spectrum just to the red side of the α band is much brighter than the area of the spectrum just to the green side. This lack of symmetry was approximately compensated for by means of the photographic plate which was used, for the latter being of the orthochromatic type is relatively insensitive to red light.

The spectro-camera which is described in detail elsewhere (4) consisted of slit of 2·7 mm. width and 7 mm. length with collimator of 500 mm. focal length. A direct vision prism grating was designed in such a way that there was projected on to the plate a reference mark which occupies a fixed position in reference to the spectrum which is projected on to another portion of the same plate during the same exposure. By means

of this reference mark it is possible subsequently to ascertain the wave length of different parts of the photographs of the spectra. The camera lens also had a focal length of 500 mm. The distance between the sodium lines (5893 Å.U.) and the thallium line (5351 Å.U.) of the spectrum was 26·5 mm., *i.e.* the dispersion in this part of the spectrum was approximately 22·2 Å.U. per mm.

The light source employed was a fullolite lamp which was supplied with direct current at 105 volts from a set of accumulators. The solutions to be photographed were placed in suitable glass cells which were placed between the light source and the slit of the spectro-camera. The light also passed through a correcting colour filter (prepared by Hartridge's method) which cut off the short wave length part of the spectrum below 5300 Å.U. The plate in its dark slide was attached to a repeating back so that a considerable number of spectra could be recorded on the same plate.

Usually photographs of spectra of solutions of known composition alternated with spectra of which the composition, was the subject of determination. These were taken on the same photographic plate because as is well known the sensitiveness of colour plates varies somewhat even between different plates taken from the same box.

The duration of the exposure was controlled by a shutter which was interposed between the prism grating and the camera lens.

The plates after exposure were developed with metol hydroquinone and were fixed, washed and dried in the usual manner.

In a recent experiment successful photographs were obtained with an Ilford Chromatic plate, H. and D. value = 135. Blood solution 1 in 200 blood, in cell 3·2 cm. thick. Exposure = 1 min. Development = 3 min.

The Measurements of the Spectra.

There appear to be at least three methods of performing analysis on the photographs of the spectra. (A) To ascertain the density at suitable points of the plates. (B) To employ the coincidence method by superposing the plate over a strip or band of the opposite density arrangement. (C) To employ the coincidence method with similar parts of the same plates (or of positives taken from them) in juxtaposition.

Unfortunately our own work was interrupted by other engagements at the moment when we were ready to begin analysing the plates and we have only had personal opportunity to test method (B). This method has given encouraging results, which will be referred to briefly at the end of this paper. At this stage, however, it happened that Dr Hecht and

Mr Morgan were in urgent need of a photographic method of determining the ratio of oxyhæmoglobin to carboxyhæmoglobin for the accurate measurement of the dissociation curve of CO-hæmoglobin. Method (A) was consequently studied by them in detail, and the particular procedure finally adopted was tested by them most thoroughly. We desire to offer to them and to Mr Forbes, who continued to use the method, our warmest thanks for allowing us to describe in this section the method of analysis which has been employed by them and proved to be entirely serviceable.

The densities of different parts of the plates were measured by an adaptation of the Watson densitometer. This instrument consists essentially of a selenium cell, the variations of resistance of which are ascertained by means of a mirror galvanometer. Two beams of light may be caused to fall one at a time on the selenium cell; one of these has passed through a chosen part of the plate which is being measured, whereas the other has passed through a standard neutral tint wedge which varies in density from one end to the other. This wedge is shifted by trial and error until the light through it gives the same galvanometer deflection as the chosen part of the plate does. The position of the wedge is now recorded by vernier reading and another part of the plate is selected for measurement.

Fig. 1 and Table I show the results obtained in a typical experiment, in which five different optical mixtures of oxyhæmoglobin and CO-hæmoglobin were photographed on the same plate. Readings with the densitometer were taken at every millimetre over the part of the plate on which the α band lies, the total length of the plate thus measured being 4 to 6 mm. With a later type of densitometer readings at every half millimetre have been taken.

TABLE I. Densitometer vernier readings of density.

Mm. above reference line at which the density is read	0 p.c. COHb Photographed as 100 p.c. O_2Hb	100 p.c. COHb	25 p.c. COHb	50 p.c. COHb	75 p.c. COHb
18		56·2	—	—	—
19	—	54·4	—	—	59·1
20	—	53·5	63·4	58·7	57·3
21	59·7	53·8	57·9	55·8	56·1
22	52·9	55·6	54·0	54·1	56·2
23	50·2	59·0	52·2	53·7	57·6
24	50·0	—	52·0	54·3	60·1
25	50·8	—	53·3	56·2	—
26	53·1	—	55·8	59·8	—
27	57·0	—	59·9	—	—

Several different methods of determining the proportions of oxy-hæmoglobin to carboxyhæmoglobin were tested with the aid of these curves. Of these two may be mentioned:

(A) Direct measurement of the shift of the apex of the α band.

The apex shifts about 60 Å.U. (or 3·5 mm. on the plate) when CO replaces O_2 from combination with Hb. Owing, however, to the flatness

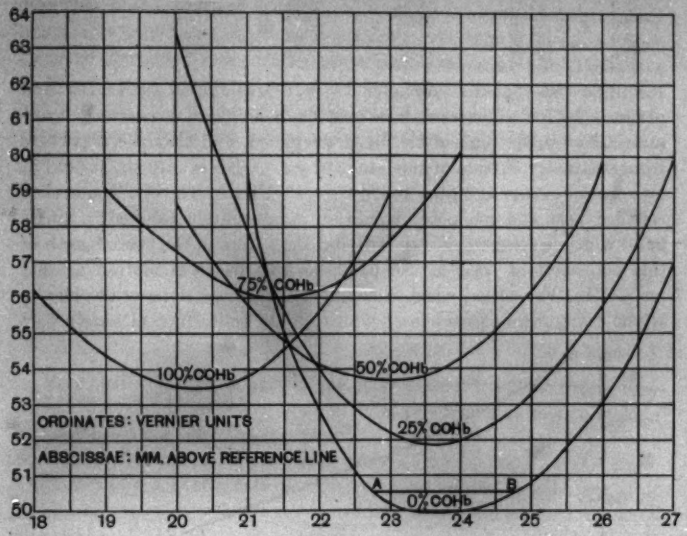

Fig. 1.

of the density wave length curve in the neighbourhood of the apex, the exact determination of the wave length of the apex offers very great difficulty. This method, therefore, proved to be impracticable.

(B) The measurement of the wave length of the mid-points of the band at a suitably selected band width.

In Fig. 1 the line AB drawn parallel to the abscissa axis intersects the curve for 0 p.c. COHb in two points A, B of equal blackness (represented by the corresponding ordinate of 50·5 vernier units) distant 22·77 mm. and 24·77 mm. from the reference mark respectively. In this case the breadth of the band, 24·77−22·77 = 2 mm., and the mid-point of the

band at this breadth of 2 mm., is distant 23·77 mm. from the reference mark.

Similar results for other band widths and curves are given in Table II.

TABLE II. Wave length of mid-point of band in mm. from reference mark.

Band width mm.	0 p.c. COHb	25 p.c. COHb	50 p.c. COHb	75 p.c. COHb	100 p.c. COHb
2·0	23·77	23·66	22·92	21·45	20·21
2·5	23·84	23·65	22·92	21·43	20·20
3·0	23·87	23·66	22·92	21·42	20·18
3·5	23·92	23·67	22·92	21·40	20·16

It will be seen that the curves for 25 p.c. COHb and 50 p.c. COHb (and presumably for all intermediate mixtures) are almost perfectly symmetrical over the part of the band examined, and that the departure from symmetry outside of this range of p.c. COHb is only slight, and of an opposite nature at higher and low p.c. COHb respectively. Being thus satisfied that the symmetry condition is adequately fulfilled, a single band width is selected as standard and the values of the wave length of this mid-point of each of the bands at this width is plotted against p.c. COHb. The actual width is so selected that the slope of the curves at the extremities of the chosen width should be inclined at nearly 45°[1]

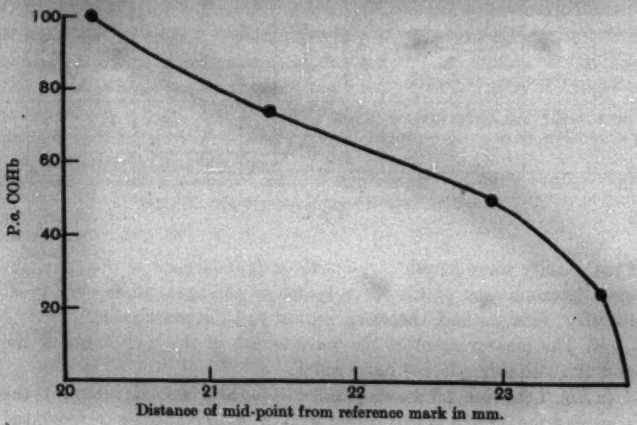

Fig. 2.

[1] Alternatively the average of the values of the mid-point of the band for each p.c. COHb might have been used.

to the axis in the case of all the curves, when the abscissæ and ordinates are plotted on the same relative scale as that used in Fig. 1. Thus in the present example the band width of 2·5 mm. is suitable. The curve so obtained is shown in Fig. 2, and may be described as the Calibration Curve of the plate.

Accuracy of the method. With careful attention to experimental details (such as the cleanliness of the solutions to be photographed) duplicate photographs of the *same* solution on the *same* plate give mid-point wave lengths which do not in extreme cases differ from one another by more than 0·033 mm., *i.e.* about 0·9 p.c. of the total shift. Reference to Fig. 2 shows that an error of this magnitude in photographing an unknown solution would cause an error in the estimate p.c. COHb which varies according to the actual range of p.c. COHb within which the unknown lies.

TABLE III.

Range within which unknown solution lies (p.c.)	Approximate error (p.c.)
0–25 COHb	6
25 „ 50 COHb	2
50 „ 75 „	0·6
75 „ 100 „	1

Since individual photographic plates, even from the same batch, vary in sensitivity, it is necessary in order to obtain this accuracy to include on *each* plate not only the spectrum of the unknown solution, but also the spectra necessary for obtaining the calibration curve of the plate. The series of p.c. COHb may be obtained most readily by the method of optical mixtures, but the solutions used for the latter purpose must be identical with the unknown solution in every respect except as regards the CO- and O_2-content of hæmoglobin, and the conditions of exposure, development, etc., should be the same in the two cases. If these requirements are adhered to, the accuracy indicated in the above table is successfully achieved.

It will be observed that the accuracy is very poor in the important range of 0 p.c. COHb to 25 p.c. COHb. Higher accuracy in this range can, however, be attained in the following manner. To equal volumes of (i) 0 p.c. COHb solution, (ii) the unknown solution add equal volumes of water containing dissolved CO,[1] so that the final p.c. COHb in (i) amounts to a value in the neighbourhood of 50 p.c. COHb. Determine the actual

[1] Without allowing any of the dissolved CO to escape into the air.

p.c. COHb in both cases (with an accuracy of 0·6 p.c.). The difference in the two p.c. values (with a small correction for the difference in the amount of dissolved CO in the two cases) gives the p.c. COHb in the unknown with an accuracy of $2 \times 0{\cdot}6 = 1{\cdot}2$ p.c., instead of 6 p.c.

The advantages of a symmetrical wave length density curve. The importance of securing a symmetrical wave length density curve has been emphasised more than once, and it is now appropriate to summarise the advantages in favour of this policy.

(i) Consideration shows that the wave length of the mid-point of a symmetrical band at a selected band thickness will not be affected by small technical errors, such as slight fogging of the plate, slight inaccuracy in adjusting the strength of the hæmoglobin to be photographed, or slight errors in the time of exposure or development.

(ii) Variations in the sensitivity of different parts of the same plate may amount to as much as 7 p.c., but such variations are only likely to occur as between widely separate regions. A symmetrical band would not be affected unless it was so wide that one side of it fell on a part of very different average sensitivity to that on which the other side of the band falls. This contingency therefore is not likely to be a source of trouble in the various bands dealt with in this paper.

(C) *The coincidence method by superposition.*

This method is in principle identical with the one just described (B). For the plate is placed face downwards on a sheet of paper on which has been inscribed a black strip of selected width. The plate is now adjusted in position by means of a micrometer until this black strip occupies the centre of the light strip on the plate which has been produced by photographing the α band. The micrometer is now used to complete the adjustments so that to the eye the two parts of the band just adjacent to the edges of the black strip appear to be of equal density. With suitable calibration the micrometer scale indicates the wave length of the mid-point of the band.

In the previous method (B) two points of equal density are found where the band has a selected width by means of a density meter containing a selenium cell. The wave length of the mid-point between these two points is then ascertained. In this method (C), on the other hand, the eye is used for making a similar selection. This method gave under test very promising results. With a total difference of position of the plate of 3·72 mm. between the α bands of oxy- and CO-hæmoglobin (60 Å.U.) the difference between the average values of ten readings on the same

band of two observers was 0·033 mm. The accuracy is therefore approximately the same as that given by method (B).

This method could most probably be improved still further by bringing corresponding parts of the two opposite sides of the band into juxtaposition so that the eye could make a more direct comparison and therefore judge more readily when the two sides are of equal density. This arrangement could be effected without difficulty by means of an internal reflecting right-angled prism mounted as in Brewster's pseudoscope.

It may be noted in conclusion that the above method has two advantages over the densitometric method. The time required for making the observations runs only into minutes instead of into hours, and the cost of the apparatus is but a small fraction of the cost of the densitometer.

(D) *Coincidence method by juxtaposition.*

In this method the plate is duplicated one half being placed in a reversed direction to the other half. This duplication and reversal enabling the same principle to be applied to the plate as that on which Hartridge's reversion spectroscope is based.

The duplication and reversal can be effected either by optical means or by cutting the plate up (or positives taken from it). This method has not been explored in any detail, but it would seem to be subject to the same limitations as those that apply to the reversion spectroscope, except that (*a*) a permanent photographic record is obtained which can be remeasured at any time, and that (*b*) the difference in colour on either side of the *a* band is eliminated.

SUMMARY.

A method is described for obtaining photographs of the absorption spectra of O_2-hæmoglobin, CO-hæmoglobin or mixtures of the two pigments. The plates are analysed at leisure either by the selenium densitometer or by a visual apparatus: in each case the position on the plate of the mid-point of the *a* band is determined to within about 0·03 mm. A calibration curve relating p.c. COHb to position of mid-point of *a* band is obtained by photographing a series of known mixtures of COHb and O_2Hb, and the p.c. COHb in an unknown solution can be obtained by recording and analysing its photograph on the same plate as was used for the calibrating exposures. The difference between the respective positions of the *a* band in the case of 100 p.c. O_2Hb and 100 p.c. COHb amounts to about 3·5 mm.; the accuracy of the method is accordingly higher than that given by the reversion spectroscope, and has the

further advantages of giving a permanent record and of being far freer from subjective errors. It is important, however, that the solution to be photographed should be free from turbidity.

Our thanks are due to the Medical Research Council for defraying, in part, the expenses involved in this research.

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The physical and physiological effects of radiant heating by the panel system. By H. M. VERNON and M. D. VERNON.

A new method of heating buildings, called the panel system, has recently come into favour. Coils of steel pipes of half-inch internal diameter are fixed slightly below the surface in the ceiling or the walls, and a stream of hot water running through them keeps the surface at a temperature of about 38° C. Such surfaces, or panels, radiate heat in all directions, and it is claimed that this method of radiant heating by means of large surfaces at a comparatively low temperature is pleasanter and more exhilarating than that afforded by hot-water "radiators" and pipes, for with these systems most of the heating is effected by convection currents of warm air, and not by radiation.

Investigations were made at a number of large offices and other buildings (including two factories, a shop, a school, and a hospital) with installations of panel heating. The radiant temperature of the walls, ceiling and floor was estimated by means of a Moll thermopile, attached to a uni-pivot dead-beat galvanometer. It was found that whilst the walls etc., of a panel-heated room (exclusive of the radiant surface) are, on an average, about 2° C. warmer than the air temperature, those of rooms heated by hot-water radiators are about 2° cooler than the air temperature. Rooms heated by gas fires and coal fires likewise have a wall temperature about 2° above that of the air. The most striking quality of panel-heated rooms lies in the evenness of the air temperature, for there may not be a difference of more than 1° C. at any spot or level, except that the air a few inches below a ceiling panel is about 1° warmer than elsewhere. In rooms heated by hot-water radiators, on the other hand, the temperatures near the ceiling may be 6° C. warmer than at foot level, and the floor itself is cooler still. Also, the temperature varies according to the proximity of the heating units, and these units take up floor space, whilst wall and ceiling panels cannot obstruct.

In order to determine the effect of panel heating on subjective sensations of warmth and of air movement, the investigators first tested the atmospheric conditions in various places near to, or remote from,

the panels, and then sat down for a half-hour period, after which the sensations were recorded on an agreed scale. As the result of hundreds of observations, it was concluded that, with one slight exception, the direct radiation from the panels did not appreciably affect the senses. The numerous operatives in a shoe factory, which had wall panels, likewise failed to react appreciably. In rooms heated by a gas or a coal fire, however, the effect of the radiant heat (at a distance of 8 feet) is very distinct, and the same sensation of warmth was felt at an air temperature of 55° F. (13° C.) as in panel-heated and hot-water radiator-heated rooms at 62° F. (17° C.). This difference of effect is due to the fact that the radiation varies as the fourth power of the absolute temperature; so the excess of radiation from a gas fire over that from surrounding objects may be 1000 times more intense than the excess from a panel heated to 38° C.

The Moll thermopile, which had 80 manganin-constantan junctions, was calibrated against the radiation from a lamp-blacked copper vessel, 1 foot square. This was filled with water at a temperature varying from 4° C. below to 31° C. above that of a standard surface at air temperature. The galvanometer deflections were in close accord with the fourth power law.

The temperature gradients and kata-thermometer determinations were made by means of a tripod stand, into the head of which were screwed two hollow jointed brass rods, one of which rose to a height of 7 ft. 3 ins., and the other reached to within 6 inches of the floor. To these rods the instruments were clamped.

A method for recording the action current of a single spot of skeletal muscle without injuring any other spot. By F. BUCHANAN.

The method consists in using as leading-off electrodes, two wicks, the one of which has been steeped in normal saline, and the other in an isotonic solution of a potassium salt, kaolin being employed in each case to prevent the liquid from spreading. The potassium solutions used were 1 p.c. KCl and 1·3 p.c. K_2HPO_4, the latter salt being chosen because it is the one present in muscle fibre. The preparation was the sartorius muscle of the frog with its nerve supply, and the recording instrument the capillary electrometer. Immediately after applying the K-electrode, the muscle underlying it became galvanometrically negative to the part underlying the other electrode in the same way as if it had

been injured, and by an amount of the same order. [The potential-difference was however increased, and sometimes considerably increased, by injuring the muscle under the K-electrode.]

When the muscle is stimulated by a single break-induction shock to its upper end or to the nerve, the action-current recorded appears to be that of the single spot underlying the normal saline electrode. To test this conclusion it was necessary to make the spot of muscle under one of the electrodes definitely incapable of responding. For this purpose a tight ligature was made near the distal end of the muscle, in other preparations, and a normal-saline-wick placed beyond it to serve as the distal electrode. A similar wick was first used as proximal electrode, the demarcation current was compensated, and the action-current recorded; then a wick steeped in one of the isotonic potassium solutions was substituted and the action-current again recorded. The records show that it had been reduced at once from 2 or 3 hundredths of a volt to 1 or 2 thousandths, and that it soon disappeared altogether. After washing out the K solution, and applying once more a normal saline wick to the same spot, the action-current was restored to its original amount and might even be enhanced. Isotonic potassium solutions therefore may be regarded as devitalising temporarily, but not permanently, muscular tissue to which they are applied. Whether it is the K-ion itself or some short-lived radioactive product of it that produces the effect is a subject for further investigation.

The experiments were made more than 22 years ago with another object in view. They were interrupted at the time, and require confirmation by the more refined methods which have since been introduced. I have however made use of the results in much of my subsequent work on reflexes, but in choosing records for publication have made use of those referring to muscles in which the tendon end had been devitalised by established methods, the only exception, so far as I can remember, being the one reproduced as Fig. 18, p. 117 of vol. v of the *Q. J. Exp. Physiol.* 1912, relating to the single instance in my experience of reflex inhibition in the gastrocnemius of the frog accompanied by a positive variation.

Method of studying the ciliated epithelium of the wind-pipe.
By Leonard Hill.

The sheep's or other mammal's wind-pipe slit along the back at the membranous part, is pinned open in the horizontal position on a sheet of cork which is just immersed in a basin of Ringer's solution kept

oxygenated at about 38° C. A fine suspension powder such as silica or of graphite is then placed on the distal end of the trachea. The powder is carried towards the larynx, and the rate of transport can be measured against a centimetre scale with a watch.

If the horse's trachea is used, strips can be cut from it which lie flat on the cork when pinned out. The wind-pipe obtained from the slaughter-house just after death can be brought to the laboratory wrapped in a paper parcel and be kept in Ringer's solution in a cold room at 50° C. for one, two or even four days. The cilia will then be found actively working when the preparation is warmed to 38° C. and oxygenated. At room-temperatures the action is very slow. A rabbit's trachea makes an excellent preparation.

A suitable graphite suspension is de Haën's Hydro-Kollag 150 diluted with Ringer's solution 1 in 5. A drop of this with the aid of a pipette is placed on the mucous membrane of the trachea. The drop spreads longitudinally as it is pushed forward. In an active preparation the point of the drop may be carried along at the rate of a centimetre in 30–60 seconds. The graphite-particles may aggregate on the mucus forming a black network with longitudinal meshes, and this network be pushed along by the action of the cilia. As the membrane is cleaned from the network the black fibrils may be seen in some cases to break and coil up so that the graphite gets aggregated into clumps. Sometimes the black drop of suspension is wrapped in a gauzy veil of mucus and be so carried up through the larynx right on to the epiglottis.

In those invertebrata which feed by means of catching fine particles in water by means of cilia and mucus, it is reported that the particles are driven as aggregates into the stomach. The entanglement of dust by nets of mucus appears to be part of the mechanism for cleansing the breathing passages by the cilia. In the roof of the frog's mouth and œsophagus aggregation of the graphite suspension similarly takes place, and black strings of mucus are driven into the stomach.

The cilia of a frog's gullet easily carry pieces of the frog's stomach along. The cilia of the gullet of a bull frog are capable of moving a well greased 5 grm. weight along the gullet at the rate of 1 cm. in about 40–50 secs. over and over again. They can even move slowly a 15 grm. weight.

The cilia in a mammal's trachea will shift a small piece of tissue but not heavy substances: they can move powder along actively; heavy powder such as calomel is carried as quickly as a light powder. Powders which flocculate are moved with greater difficulty. Mercuric oxide powder

and lime poison the cilia. The arrest of the graphite suspension at an area thus poisoned or killed by a cautery can be studied. The cilia of the trachea are remarkably resistant to ether, chloroform and alcohol.

The digestion and absorption of fat. By J. MELLANBY.
(*Preliminary communication.*)

The accepted hypothesis for the absorption of fat from the alimentary canal assumes (1) that fat is taken into the columnar cell of the villus as fatty acid and glycerol, (2) that re-synthesis of fat occurs in these cells, and (3) that neutral fat passes from the cell into the villus spaces, and thence into the central lacteal. On this hypothesis neutral fat can pass from the cell into the villus spaces, whereas absorption into the cell through the intestinal border requires previous hydrolysis of the fat.

Experiments on cats indicate that fat does not require to be hydrolysed into fatty acid and glycerol by lipase before intestinal absorption takes place.

The amount of lipase in cats' pancreatic juice is very small and the enzyme is practically absent from the juice secreted after the first 3 c.c. An examination of the alimentary canal of the cat shows that there is no additional source of lipase. Hence the quantity of fat capable of being hydrolysed in the alimentary canal of a cat is small. In accordance with these observations it is found that emulsified fat does not require previous hydrolysis before absorption by the villi of the small intestine.

Cats (fasting for the previous 16 hours) were anæsthetised with veronal and cannulæ were inserted into the gall bladder, the common bile duct and the pancreatic duct. During the experimental observations the secretions of bile and pancreatic juice were collected and fat absorption determined by inspection of the lacteals in the mesentery.

(1) The injection of emulsified fat into the duodenum leads to no absorption of fat, no secretion of pancreatic juice and no change in the secretion of bile.

(2) Similar negative results are obtained when emulsified fat, previously digested with an active lipase, is injected into the duodenum.

(3) The injection into the duodenum of emulsified fat to which bile has been added causes (*a*) the rapid absorption of fat into the lacteals, (*b*) the secretion of pancreatic juice, and (*c*) an increased flow of bile.

(4) The injection into the ileum of a similar mixture of fat and bile causes (*a*) a slow absorption of fat into the mesenteric lacteals, (*b*) an increase in the flow of bile, and (*c*) no secretion of pancreatic juice.

The relation of the absorption of bile to the flow of pancreatic juice has been analysed in a previous paper. The observation which I wish to emphasise is that a mixture of neutral fat and bile only, to which no lipase has access, is rapidly absorbed from the duodenum and jejunum and less rapidly from the ileum into the lymphatic system of the small intestine.

The histological appearances of the cells of the villi indicate that fat passes in a very finely divided state (possibly molecular) through the columnar cell and appears in the intracellular spaces and central lacteal of the villus as very fine particles.

Possibly the main function of the lipase of pancreatic juice is to hydrolyse a small quantity of fat into fatty acid and glycerol, and the soap formed by the neutralisation of this fatty acid by the alkali of the pancreatic juice facilitates the formation of a fine emulsion which is essential to rapid absorption when admixed with bile.

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July 23, 1927.

Studies in the metabolism of the bile acids. By CARL H. GREENE, MARTHA ALDRICH and L. G. ROWNTREE (*Mayo Clinic, Rochester, Minnesota*).

The studies of Stadelmann, Whipple and others on the metabolism of the bile acids are well known. Because of the limitations of the methods used, their studies were confined to the changes in the bile and urine. By a quantitative adaptation of the Pettenkofer reaction, glycocholic acid in amounts of from 0·1 to 0·5 mgm. can be determined with an error of approximately 5 p.c. This reaction has been applied to the alcoholic extract of 5 c.c. of blood and the method of extraction selected to permit the determination of maximal amounts of the Pettenkofer reacting material. Normal blood gives a colour equivalent to that from 3 to 6 mgm. of glycocholic acid per 100 c.c. Bile acids added to blood may be recovered with an average loss of but 0·5 mgm. per 100 c.c. In general the recovery is better than 90 p.c.

The method can be applied to bile and its accuracy has been tested by comparison with that of Schmidt and Dart. The values obtained for the analysis of bile by the Pettenkofer method agree well with those obtained by the gasometric determination of the amino-nitrogen liberated by alkaline hydrolysis. When only small amounts of material are available, or when the ease and rapidity of analysis enter into the choice, this modification of the Pettenkofer reaction would seem to be the preferable method. By it we have been able to follow certain phases of the metabolism of the bile acids, more closely than has been possible heretofore.

When whole bile or bile salt preparations are injected intravenously the bile acid content of the blood increases markedly. The maximal value attained depends on the dose given and the speed of the injection. After such an injection the bile acids leave the blood rapidly. Even with maximal doses the excess is removed within two hours.

The excretion of bile acids by a dog with a biliary fistula has been studied and the curve of elimination from the blood compared with the curve of excretion in the bile. The rapidity of excretion of the bile acids

and the independence in the manner of excretion of the bile acids and bilirubin is re-emphasised.

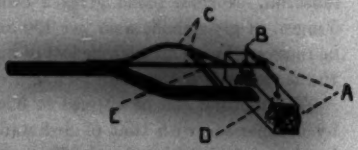
When bile or bile acids are given by mouth, the amount in the blood of the portal vein is increased though the level in the systemic circulation (jugular vein) is unchanged. This speaks for the entero-hepatic circulation of the bile acids.

The bile acid content of the blood increases rapidly following experimental obstruction to the common bile duct. After the first few weeks of obstructive jaundice the values decrease and tend to return to the normal level indicating probable failure of the synthesis of the bile acids. The rate of elimination of intravenously injected bile acids from the blood stream is greatly delayed in the presence of experimental obstructive jaundice.

A simple adjustment to deliver make induction shocks.

By N. E. Condon (*Edinburgh*).

The standard pattern of induction coil can easily be modified so as to short circuit the break shock from the secondary coil when single induction shocks are employed.



A. Terminals. *B*. Platinum wire.
C. Fine watch spring, upper piece to check.
D. Vulcanite block, containing two mercury cups.
E. Piece of flattened soft iron wire.

The arrangement is shown in the diagram. The general principle is the same as that devised by Laidlaw (*Journ. of Pharm. and Exp. Ther.* 5. p. 469. 1913), but is a simplification of this apparatus. The platinum wire *B* which short circuits the secondary break shock must be insulated from the spring *C* by which it is carried. Any induction coil can be quickly altered from the ordinary pattern to the pattern described, since it is only necessary to remove the hammer from the coil, to slip into position the apparatus and then to connect in the usual manner, except that the extra connection shown in photograph is essential.

A simple slide rule for the calculation of respiratory quotients.

By W. A. M. SMART.

The slide rule here described has been designed for the rapid determination of the Respiratory Quotient, from experimental data of the percentage composition of the inspired and expired air.

Formula for the Respiratory Quotient. Let x and X represent the percentages of carbon dioxide in the expired and inspired air respectively, while y and Y stand for similar values in regard to oxygen. Since the volume of carbon dioxide given off is not equal to that of the oxygen absorbed, it follows that the percentages of oxygen (or carbon dioxide) inspired and expired cannot be directly subtracted. It is therefore necessary to correct one or other of the inspired or expired percentages for this difference in volume. In strictness, the full formula for the Respiratory Quotient under any atmosphere of oxygen, carbon dioxide and nitrogen only, may be written,

$$\text{R.Q.} = \frac{x - X\left(\frac{100 - x - y}{100 - X - Y}\right)}{Y\left(\frac{100 - x - y}{100 - X - Y}\right) - y}.$$

This generalised formula can, however, be considerably simplified in practice. In the first place, the corrected reading of the inspired carbon dioxide, viz. $X\,(100 - x - y/100 - X - Y)$ differs so slightly from that of the uncorrected, being in the fourth decimal place, that it may be neglected. Secondly, if we adopt standard values of the inspired air, two of the variables become constants, thereby making the formula very much simpler. We take inspired air as having the constant percentage composition, oxygen 20·93 p.c., nitrogen 79·04 p.c., and carbon dioxide 0·03 p.c. Using these values the formula reduces to

$$\text{R.Q.} = \frac{x - 0{\cdot}03}{20{\cdot}93\left(\frac{100 - x - y}{79{\cdot}04}\right) - y}.$$

By putting $\lambda = 20{\cdot}93/79{\cdot}04 = 0{\cdot}2648$ this still further reduces to

$$\text{R.Q.} = \frac{x - 0{\cdot}03}{\lambda\,(100 - x - y) - y}.$$

Though the formula has thus been considerably simplified, it is still a tedious matter having to use it repeatedly during the course of a set of experiments. To avoid this, a slide rule has been devised, whereby, knowing the experimental values of oxygen and carbon dioxide percentage in the expired air, that of the respiratory quotient can be found immediately.

Description of the Instrument. The upper scale of the slide rule has been graduated to read directly the values x of the percentage of expired carbon dioxide. The slider is graduated to read the values y of the percentage of oxygen in the expired air. The lower scale gives the corresponding value of the respiratory quotient. To use the ruler, set the slider so that the graduation representing the observed value of y lies collinear with that of the observed reading of the percentage content x of carbon dioxide. The arrow will then automatically point to the resulting value of the respiratory quotient.

The drawing (on a reduced scale) shows the instrument set for the values of $x = 4{\cdot}70$ and $y = 16{\cdot}60$, giving a respiratory quotient of 1·10.

Theory of the Instrument. For those who may be interested, the following is the theory of this particular slide rule:

Multiply out the above formula and we have

$$\text{R.Q.}\ \lambda\,(100 - x - y) - y.\text{R.Q.} = x - 0{\cdot}03.$$

Now put $X \equiv x - 0{\cdot}03$ giving

$$\text{R.Q.}\ \lambda.100 - \text{R.Q.}\ \lambda.y - \text{R.Q.}\ \lambda\,(X + 0{\cdot}03) - y.\text{R.Q.} = X$$

$$\text{R.Q.}\ (100\lambda - \lambda y - y + \lambda\,0{\cdot}03) = X\,(1 + \lambda\,.\,\text{R.Q.})$$

$$(100\lambda - \lambda y - y + \lambda\,0{\cdot}03) = X\,(1/\text{R.Q.} + \lambda)$$

Again, if we put $Y \equiv 100\lambda - y\,(1 + \lambda) + 0{\cdot}03\lambda$ and $Z \equiv 1/\text{R.Q.} + \lambda$ the above reduces to $Y = X.Z$ which is readily adapted to slide rule form. The upper scale can be taken to represent X, the slider Y, and the lower scale Z. When the ruler is graduated (in the orthodox way by logarithmic scales) it is an easy matter to label the various readings of X, Y and Z, with those of the x, y and R.Q., from which they were derived. The arrow may of course be placed in any convenient position.

Effect of ether anæsthesia on some blood constituents. By H. E. Magee and Miss A. E. Glennie (*Rowett Institute, Aberdeen*). (*Preliminary Communication.*)

The influence of anæsthesia on the Ca and P of blood is not well established. In the course of some experiments where it was necessary to estimate these constituents in the blood of animals under ether anæsthesia, we observed that the ether apparently had definite effects

upon them. We therefore thought it desirable to determine (1) the effect of ether anæsthesia on the Ca and P of blood and (2) whether the changes observed were related to the well-known effects of anæsthesia on the sugar and *p*H.

Rabbits which had fasted 15 hours were used. Blood was drawn just before and every 15 minutes during the anæsthesia and the *p*H (method of Martin and Lepper), P (Briggs-Bell-Doisy method) and sugar estimated on each sample. The Ca estimations (method of Kramer and Tisdall) were carried out in separate experiments on the serum. The corneal reflex was always kept negative during the anæsthesia. The following table shows the nature of the results obtained.

Results.

Rabbit No.	Mins. under ether:	0	15	30	45	60
1	*p*H	7·30	7·14	7·15	7·20	7·23
	P mgm. p.c.	4·28	5·91	6·01	5·54	5·49
	Sugar mgm. p.c.	181	241	290	412	383
10	*p*H	7·35	7·10	7·15	7·18	7·23
	P mgm. p.c.	3·10	6·98	6·90	6·66	5·83
	Sugar mgm. p.c.	250	362	369	534	578
28	*p*H	7·30	7·03	7·06	7·12	7·20
	P mgm. p.c.	4·93	7·18	8·56	7·71	7·64
	Sugar mgm. p.c.	154	210	253	236	286
Ca mgm. p.c.	(Av. for 5 rabbits)	14	13	13	13	12

The results show that the *p*H fell after 15 minutes anæsthesia and then rose slowly, but never to the initial value. The fall was not due to anoxæmia, for similar results were obtained when pure O_2 and ether were given. In nearly every case the P was definitely increased, sometimes progressively like the sugar, but more often the level reached after 15 minutes was maintained fairly steady. In a very few cases there was no definite increase in P but there was never a fall. The serum Ca was lowered slightly but consistently by the anæsthetic.

The action of acetyl choline on the cardiac frequency and the blood-pressure of the cat. By A. J. Clark and A. C. White (*Edinburgh*).

Acetyl choline has been found by one of the authors (A. J. C. (1)) to show a characteristic relation between concentration and action on the frog's tissues. The relation is expressed by the formula $K.x = \frac{y}{A-y}$ where x = concentration, y = action, A = maximum action produced by the highest concentrations employed and K = constant.

We made experiments to see if this relation held true when the drug was given to the intact cat. The cats were pithed and the drug injected intravenously at a constant rate in a constant volume of solution.

The action of the drug on the heart rate was found to follow the above formula fairly closely as is shown by the following example. In the calculated reduction in rate shown in the last line, $K = 50$ and $A = 95$ p.c.

Dose in mgm. per kilo $\times 10^8$	0·425	1·4	4·25	14	42·5	140	425	1280	4250
Rate before injection	214	220	215	220	214	210	225	205	205
Minimal rate after injection	214	208	190	126	93	40	c.10	c. 10	c. 10
Reduction in rate, expressed as p.c. of initial rate:									
(a) Observed	0	5·5	12	43	56	81	95	95	95
(b) Calculated	2	6·2	16·6	39	64·5	83	91	94	95

The agreement between the observed and calculated figures is fairly good since there are various unavoidable errors. For instance any change in the minute volume of the heart alters the rate at which the drug reaches the pacemaker.

The drug has a much more powerful action on the blood-pressure than on the rate. We found that a dose of 4×10^{-6} mgm. per kilo produced a measurable fall in blood-pressure. (Hunt and Taveau(2) obtained a response with one-hundredth of this quantity.) A graded response was obtained over nearly a millionfold range of dosage, but unfortunately doses above 10^{-4} mgm. per kilo produced a partly irreversible effect. The following figures were obtained and they show the extraordinary range of dosage over which the drug produced a graded response.

Dosage in mgm. per kilo $\times 10^8$	Blood-pressure in mm. Hg		P.c. reduction in blood-pressure
	Before	After	
1·4	70	70	0
4·25	72	65	10
14	75·6	59	21·5
42·5	77	55	29
140	74	50	32·5
425	68	46	32·5
1400	60	42	30
4250	54	38	30
14,000	52	32	38·5
42,500	59	32	46
140,000	50	22	56
425,000	41	16	61
1,200,000	36	16	56
4,250,000	32	16	50

It is interesting to note that in the case of two tissues in the same

animal, namely, the arterioles and the pacemaker, the former are more than one hundred times more sensitive to acetyl choline than is the latter.

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2. Hunt and Taveau. Journ. of Pharm. and Exp. Ther. 1. p. 303. 1909–10.

A method for showing continuous tracings on the screen.

By N. E. Condon (*Edinburgh*).

This apparatus was devised to enable long tracings to be shown to a large class. Straub's "Kurvenkino" has the disadvantage that it will only show fairly short tracings. In my apparatus a film is wound from a spool across the field of a lantern on to a small drum revolved by clockwork (Fig. 1). The film is $2\frac{1}{4}$ inches in width, and films several yards in length can be used.

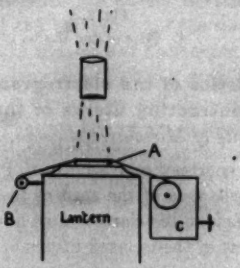


Fig. 1.

A. Piece of plate glass to prevent curling of film.
B. Ordinary Kodak film spool, drilled, to run on stand or bracket.
C. 2″ cylinder run by clockwork, to receive film.

Tracings can be taken direct on to bare celluloid film which has been smoked and stretched round a kymograph, whilst larger tracings can be photographed on to plates and these printed in series on a length of sensitised film.

Care is required in smoking celluloid films, but this can be done safely by interposing a frame of a double thickness of $\frac{1}{8}$-inch mesh wire gauze between the flame and the film. In varnishing the film must be run through the varnishing solution slowly, considerably slower than is usual for paper tracings, otherwise the carbon is washed off the film. A suitable record gives the illusion that recording levers are

writing on the screen. To obtain this effect suitable speeds for recording and showing the film must be selected. Moreover the edge of the screen where the film first appears must be perfectly sharp and vertical. Pasting a strip of lantern slide binding on the extreme edge of the piece of plate glass, inserted to prevent the film from curling, provides a suitable edge. This is best done while the lantern is on. Frontal levers are necessary and for fast work writing points as in Fig. 2 have been found satisfactory. The film used is cheap, and once a good film is obtained, it can be used over and over again for demonstration purposes, as the film surface is not easily scratched.

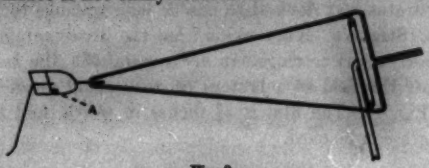


Fig. 2.
A. Piece of fine elastic.

Unique characteristics of the electrogram of the isolated and automatically contracting uterus of the rat. By CHARLES W. GREENE. (*University of Missouri, Columbia, Missouri.*)

By the generous courtesy and advice of Professor Einthoven and with the technical assistance of the staff of the Physiological Institute of the University of Leiden bio-electrograms have been obtained during automatic contractions of the isolated uterus of the rat. The absence of previous reports in the literature and the presence of new and unusual features in the electrogram of the uterus as compared with that of other organs justifies a preliminary report.

The method has been to record simultaneous electrograms and myograms from the isolated uterine horn contracting automatically in a moist chamber. The tissue was moistened with Ringer's solution and the temperature was maintained at 34° C to 36° C. The mechanical contractions were recorded by very light and counterpoised Einthoven levers. The action currents were led off by Porter nonpolarisable electrodes placed so that negativity at the ovarian end of the tube produced a positive deflection in the photographic record.

The preliminary results are briefly summarised as follows:

1. Isolated uterine horns of the rat suspended in air in a warmed moist chamber develop automatic rhythmic contractions. These persist

for long periods and are similar in type to the automatic contractions of preparations mounted in warmed aerated Ringer or Ringer-dextrose solutions.

2. Bio-electric changes in potential occur in such preparations simultaneously with the mechanical contractions. The action currents so developed appear with the automatic contractions, and the preparation becomes isoelectric during the pause between contractions.

3. Two types of electric deflections occur in the rat's uterus: First a slowly developed simple deflection which increases to a maximum during the contraction phase of the simple mechanical contraction, and gradually disappears with the mechanical relaxation. This form of electrogram has been shown to occur in a variety of other smooth muscle organs.

4. The second type of action current observed in the rat's uterus seems not to have been described previously. It consists of intermittent or periodic electric discharges during the mechanical contraction phase only. It is compounded with the slower deviation of the first type. In our least complicated and best record of the simple electrogram there are thirty-six deflections in the series of discharges. The deflections begin as scarcely perceptible deviations, become stronger and more rapidly repeated for three or four discharges, and then continue with constant intensity and rhythm. The last five or six deviations again decrease in intensity and rate to completely disappear at the crest of the mechanical wave. The first discharge occurs from 0·5 to 0·8 seconds before the mechanogram begins. This delay of the mechanogram can be looked upon as a latent period. The regularity of the rhythm is indicated by measurements of the time intervals in seconds in one example: 0·80, 0·60, 0·56, 0·54, 0·52, 0·52, 0·52, 0·52, 0·52, 0·52, 0·50, 0·50, 0·54, 0·50, 0·52, 0·50, 0·52, 0·56, 0·52, 0·56, 0·58, 0·56, 0·52, 0·52, 0·52, 0·54, 0·56, 0·60, 0·66, 0·68, 0·68, 0·76, 0·82, 0·82, 1·40.

5. The individual deflections of the second type deviate only slightly from simple positive monophasic waves like the P-wave in the normal electrocardiogram. However, the positive deflection is sometimes preceded by a very brief, inconstant and faint negativity. The earlier deflections of a series return to the isoelectric base line rather slowly. Later in the series equilibrium is established more promptly.

6. Complex records of a great variety of types have been obtained. These can be explained with satisfaction on the view that simultaneous simple contractions occur with various degrees of compounding.

The first or slow deflection in the utero-electrogram is a simple myo-

electrogram, typical of the contractions of smooth muscle of hollow organs everywhere.

The interpretation of the second form of deviation is more difficult. The evidence of a rapid rhythm-production indicates the presence of an apparently new factor in the activation of such organs. The relation of the electrical change to the active period of the mechanical contraction suggests an analogy to the production of tetanus in voluntary muscle by periodic nerve stimulation. The origin and significance of these physiological phenomena are being studied in detail.

The purification of the lactic acid forming enzyme of muscle.

By Otto Meyerhof and K. Meyer.

The lactic acid forming enzyme extracted from frog or rabbit muscle can be purified from other extractives by bringing the extract to *p*H 5 with acetate buffer and thereby precipitating the protein and enzyme. On subsequent elution with diluted phosphate at *p*H 8 the ferment is redissolved while a large fraction of the protein remains in the precipitate. This process is repeated, after which the activity has risen from a production of 0·04 mgm. lactic acid per mgm. protein and hour (at 20° C.) by the fresh extract to 0·5–0·9 mgm. The activity may be further increased by adsorption on $Al(OH)_3$, made according to Willstätter's method, to 1·0–1·5 mgm. carbohydrate split per mgm. protein and hour, and remains constant on further elution with dilute phosphate.

The coenzyme and all phosphoric acid esters are removed during the purification, so that boiled muscle juice must be added before the above described glycolysis is obtained. When, in the boiled juice, the coenzyme is separated from the hydrolysable phosphoric acid esters, the system is no longer able to split glycogen or esterify it with phosphate. Besides enzyme and coenzyme a hydrolysable ester is necessary; this can be recovered from the baryta precipitate of fresh muscle extract or boiled juice. This substance is not "lactacidogen," the hexose-monophosphoric ester of Embden, which has been isolated from muscle by Dr Lohmann in another way than that recently described by Embden, but is another ester which behaves as a hexose-diphosphoric ester. This corresponds to the rôle of hexose-diphosphoric acid in inducing alcoholic fermentation in yeast juice.

On purification, the lactic acid enzyme becomes more stable and retains its activity for several days.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
October 15, 1927.

The concentration of CO_2 in successive portions of an expired breath.

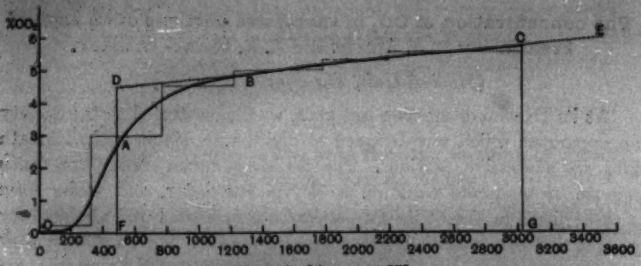
By R. S. Aitken and A. E. Clark-Kennedy.

(*Medical Unit, The London Hospital.*)

At its December meeting last year, we demonstrated to the Society an apparatus which was designed to divide up a single expired breath into six successive portions. The breath is taken during the tenth minute of moderate work on a bicycle ergometer, and varies from 2 to 3½ litres in size. The six portions are measured and analysed for oxygen and CO_2, and their CO_2 concentrations are plotted against their volume; a smooth curve is drawn through the rectangular steps representing the successive portions, as shown in the figure.

A considerable number of experiments of this kind has been done. It is found that the experimental curve has a typical form: an S-shaped rise from the origin up to 1000 or 1200 c.c., followed by a straight portion sloping gently upwards. The S-shaped portion represents alveolar air diluted with fresh air from the dead space (instrumental plus physiological); the straight portion represents undiluted alveolar air. In this type of breathing, then, the alveolar CO_2 concentration during more than half the expiratory phase is rising at a steady rate. It seems fair to assume that during the earlier part of expiration it also rises at the same rate; in that case it can be represented by a backward prolongation of the straight portion of the experimental curve, *i.e.* by the dotted line *BD* in the figure. If the length *BD* be such that the area *FDCG* equals the area *OABCG* (each area representing the amount of CO_2 in the whole breath), then *D* indicates the alveolar CO_2 concentration at the beginning of expiration. Moreover, the distance *OF* represents the volume of the total dead space. In the same fashion, the straight portion of the experimental curve can be prolonged to the right to *E* over a distance equal to *OF*, to represent the CO_2 concentration of the air left in the dead space at the end of expiration. *DE* then represents the changing alveolar CO_2 concentration throughout this expiration; in the breaths we have investigated the rise is about 0·5 p.c. CO_2 per litre of air expired.

The mid-point of *DE* is the average CO_2 concentration during expiration. In a 3-litre breath, it is about 0·5 p.c. lower than the CO_2 concentration of the last 10 c.c. of the breath, which are regarded by Yandell Henderson(1) as average alveolar air, and are collected by his automatic sampler and by Clark-Kennedy and Owen's(2) modification of it.



The concentration of CO_2 in successive portions of an expired breath.
OABC: Experimental curve.
DE: Deduced curve representing alveolar CO_2 during expiration.
Duration of expiration: 1·77 secs.
Taken during work: CO_2 output 2100 c.c. per min. at 37°.

The physiological dead spaces obtained by the above method lie between 280 c.c. and 392 c.c., and in nine experiments on one subject they show a definite relationship to the volume of the tidal air (in the last minute of the experiment), being greater when the breathing is deeper, as shown in this table:

Exp. No.	Average Tidal Air c.c. at 37°	Physiological Dead Space c.c. at 37°
18	1890	283
20	1930	290
19	1940	280
23	2356	287
22	2371	295
24	2760	327
21	2880	361
26	3410	378
25	3520	392

These values for the dead space are somewhat lower than those which were found by Douglas and Haldane(3) during exercise; they are considerably higher than those of Krogh and Lindhard(4).

1. Henderson and Haggard. Amer. Journ. Phys. 73. p. 193. 1925.
2. Clark-Kennedy and Owen. Quart. Journ. Med. 20. p. 383. 1927.
3. Douglas and Haldane. This Journ. 45. p. 235. 1912.
4. Krogh and Lindhard. This Journ. 47. p. 30. 1913–14.

Observations upon the respiratory exchange, temperature and sugar in the blood of anæsthetised animals. By E. T. Conybeare, H. B. A. R. Densham, M. Maizels and M. S. Pembrey.

A series of observations has been made upon rabbits before, during and after anæsthesia produced by urethane or ethylene.

Sugar in the blood. The values are given in mgm. per 100 c.c.; the blood was drawn from the auricular vein.

		mgm. of sugar per 100 c.c. of blood	No. of observations	No. of animals	
Before anæsthesia	Fasting	105–152	9	9	
,,	Fed	105–200	18	18	(M.)
,,	,,	96–200	40	7	(D.)

During anæsthesia produced by urethane a rise in the sugar of the blood was observed in each case, and, as a rule, was as great in the fasting rabbits as that observed in the well-fed animals. The increase was greater when the anæsthesia was deep.

Urethane anæsthesia	Range of increase in sugar in blood: 1st hour	3rd hour	
Light	9–49	25–130	Fasting
,,	25–38	67–153	Fed
Deep	48–169	100–290	Fasting
,,	42–166	99–259	Fed

The rectal temperature fell during anæsthesia; the lowest record was 26·8° in a rabbit with the sugar of the blood raised to 456 after 24 hours' anæsthesia. As a rule the lowest temperatures were associated with the highest values for the sugar in the blood and the lowest values for the respiratory exchange.

Ethylene Anæsthesia.

Animal No.	Sugar in blood before anæsthesia	Average sugar in blood during anæsthesia	Average rectal temperature during anæsthesia	Tolerance to sugar during anæsthesia	Average rectal temperature at the time of the test for tolerance
VIII	150	152	37·5°	Decreased	34°
		305	33·4°		
IX	125	100	36·5°	Decreased	34·5°
		255	32·0°		
X	120	130	37·2°	Increased	36·5°
			35·5°	Decreased	34·6°
XIII	122	113	36·5°		
XIV	100	80	37·5°		
XII	170	157	37·0°		

When the rabbit remained under the influence of urethane the sugar in the blood was maintained at a high level during the second and third

days, even in the case of animals which had fasted before the injection of the drug.

Condition	Maximum of sugar in the blood 1st day	2nd day	3rd day	Anæsthesia
Well fed	343	284	—	Deep
"	426	260	—	"
"	316	180	110	"
"	340	290	176	Light
"	318	230	205	Deep
"	256	348	—	"
Fasting	444	430	—	"
"	313	436	260	"
"	442	366	—	"
"	282	299	—	Light
"	256	440	—	"
"	304	340	—	Deep
"	260	250	—	Light
"	323	294	—	"
"	420	340	330	Deep

A few observations have been made on the sugar passed in the urine and the amount of glycogen in the liver under different conditions of anæsthesia. One rabbit weighing 2·2 kgm. excreted 3·3 grm. of sugar in 48 hours of anæsthesia, another 3·5 grm. in 36 hours.

A large number of observations upon the influence of anæsthetics on the respiratory exchange has been made; there are special difficulties with ether and ethylene. The following is an example of the effects of urethane; the periods are for 1 hour.

Time	H_2O grm.	CO_2 grm.	O_2 grm.	$\frac{CO_2}{O_2}$	Rectal temperature	Sugar in blood mgm. in 100 c.c.	Remarks
	1·60	2·66	2·08	0·93	38·5°	131	Before injection, well-fed rabbit, weight 1·7 kilo.
After 4 hours under drug	0·79	2·24	1·77	0·91	35	230	Deep anæsthesia
23 hours under drug	0·73	1·13	1·28	0·65	35	256 300	"
28 hours under drug	1·06	1·42	1·35	0·76	31	348	"

There appears to be evidence of the production of sugar from fat.

The difference of *p*H between plasma and red cells.

By A. C. Hampson and M. Maizels.

Experiments were undertaken to determine the *p*H of the plasma and of the red cells in normal subjects and in certain cases of anæmia.

Blood was drawn from a vein and allowed to run under paraffin in a centrifuge tube. Heparin was used as an anti-coagulant. The blood

was centrifuged and the *p*H of the plasma was determined at room temperature by means of the glass electrode. The cells were laked by freezing and brought to room temperature. The *p*H was then determined.

In normal individuals the following results were obtained:

TABLE I. ($T = 20°$ C.)

	*p*H of plasma	*p*H of cells	Difference
1	7·485	7·382	0·103
2	7·490	7·398	0·092
3	7·530	7·421	0·109
4	7·535	7·421	0·114
5	7·490	7·385	0·105
6	7·500	7·415	0·085
7	7·490	7·420	0·070
8	7·472	7·421	0·051
9	7·422	7·330	0·092
10	7·490	7·370	0·120
Mean	7·490	7·396	0·094

In patients suffering from pernicious anæmia similar estimations were made and in addition, the percentage of hæmoglobin in the blood and the amount of bilirubin in the plasma was determined (Table II).

TABLE II. ($T = 20°$ C.)

	*p*H of plasma	*p*H of cells	Difference	p.c. Hb	v. d. Bergh's test
1	7·665	7·182	0·483	20	+ + +
2	7·485	7·082	0·403	50	+ +
3	7·505	7·228	0·277	20	+(+)
4	7·450	7·221	0·239	80	+
5	7·490	7·288	0·202	90	(+)

Several cases of acholuric jaundice were also examined; the fragility to hypotonic saline being also determined:—

TABLE III. ($T = 20°$ C.)

	*p*H plasma	*p*H cells	Difference	p.c. Hb	v. d. Bergh's test	Fragility to Hypotonic saline: Began p.c.	Fragility to Hypotonic saline: Complete p.c.
1	7·488	6·981	0·507	88	+ + +	0·72	0·45
2	7·420	7·012	0·408	52	+ +	0·78	0·45
3	7·472	7·120	0·352	65	+(+)	0·68	0·39
4	7·474	7·174	0·300	60	+	0·66	—

The *p*H readings were taken at room temperature, but, as C. J. Martin has shown, the correction for temperature is probably the same for cells and plasma. The difference in *p*H between cells and plasma at room temperature may, therefore, be taken as being the same at body temperature.

It will be noted that in normal subjects, the average difference of *p*H between cells and plasma was 0·094; the extremes being 0·051 and 0·120. The cells were more acid.

In five cases of pernicious anæmia, the *p*H difference varied between 0·20 and 0·48.

In four cases of acholuric jaundice, the *p*H difference varied between 0·30 and 0·50.

A further case of acholuric jaundice, not included in Table III, was examined. This case had been treated by splenectomy and had so improved as to appear almost normal. The hæmoglobin was 98 p.c., and the van den Bergh test was negative, although slight hæmolysis occurred in 0·60 p.c. saline. The *p*H difference in this patient was 0·122—hardly above normal.

In two cases of non-hæmolytic anæmia, the *p*H differences were 0·037 and 0·120 respectively.

In the hæmolytic conditions, the difference of *p*H between cells and plasma was increased as compared with the normal: this difference varying directly with the degree of hæmolysis (as estimated by the van den Bergh reaction for bilirubin), and not with the degree of anæmia, nor with the alteration of fragility of the red cells to hypotonic saline.

In the hæmolytic conditions, the increased difference of *p*H was due to the red cells being more acid; the *p*H of the plasma being within normal limits—except in case 1 of Table II, where the patient was markedly dyspnœic and the plasma was more alkaline than normal.

The effect of injections of pituitary extract, adrenalin and insulin on Ketonuria. By J. H. Burn and H. W. Ling. (*Preliminary communication.*)

Rats, weighing from 100–150 grm., have been kept in metabolism cages of the Hopkins pattern, and fed on a diet of filtered butter. Wigglesworth(1) showed that in this condition rats develop Ketonuria, which attains a maximum about the third day, and which for the most part disappears by the sixth day. The acetone bodies have been estimated in the urine by the method of van Slyke(2).

We find that the Ketonuria produced varies in amount according to the time of year. It is greatest in May and June, and least in winter. Cori and Cori(3) have observed a similar seasonal variation in the Ketonuria occurring in fasting rats.

We have found that, in May and June, injections of pituitary extract (1 unit per rat given four times a day), on the second and following days of the fat diet, greatly diminish or inhibit the Ketonuria.

Injections of adrenalin (0·04 mgm. per rat given four times a day) have the same action. All experiments in which injections were made were controlled by observations on the same animals in a second period of fat feeding after an interval of three weeks. It is not easy to demonstrate any inhibitory action of pituitary extract on the small Ketonuria which occurs in winter.

We have some evidence, which is incomplete, that a single intravenous injection of 0·3 mgm. thyroxine on the first day of the fat diet inhibits the Ketonuria also.

In surprising contrast to these effects is the effect of injections of insulin. Injections varying from 0·2 to 0·4 unit per rat have been given four times a day on the second and third days of the fat diet. These injections have been enough to produce collapse, though only rarely convulsions.

As a result, the second day Ketonuria is almost always increased, the increase being in the neighbourhood of threefold. The third day Ketonuria is also usually increased but often it is less in amount than on the second day (whereas in the absence of injections a third day maximum is the rule). When on the fourth day no insulin is given, the Ketonuria usually diminishes at an abnormally rapid rate, and remains very small on the fifth day. In the absence of more injections a second period of Ketonuria supervenes on the sixth and seventh days, disappearing again on the eighth day. This second period can be suppressed by injections of adrenalin or pituitary extract, but not by injections of insulin. The second Ketonuria is not observed in winter.

(1) Wigglesworth. Biochem. Journ. 18. p. 1203. 1924.
(2) van Slyke. Journ. Biol. Chem. 32. 1917.
(3) Cori and Cori. Journ. Biol. Chem. 72. p. 615. 1927.

The compensation by the skin vessels during over-ventilation in man. By R. A. Collier, H. B. A. R. Densham and H. M. Wells. (*King's College, London.*)

Dale and Evans found a considerable fall in systemic blood-pressure on over-ventilating anæsthetised cats. Yandell Henderson has recorded a similar change in dogs, but in man he has found that there may be little or no change in the blood-pressure.

These results having been confirmed, it seemed to be of interest to determine what factor is responsible for the maintenance of the blood-pressure in over-ventilation in man. Yandell Henderson has shown that it is not the heart, so that it is presumably the blood vessels.

Dale and Evans showed that over-ventilation of an anæsthetised cat is associated with an increase in the volume of its limbs. Nahun showed that there is a decrease in limb volume when a normal man over-ventilates; while Stewart found a decrease in the circulation through the hand, and Wells found a fall in the electrical resistance of the skin under the same circumstances.

It appeared then that the vessels of the skin play some part in maintaining the blood-pressure during over-ventilation in man. This hypothesis seems largely confirmed by the fact that, in an individual whose blood-pressure normally rises on over-ventilation, there is a fall in blood-pressure when the skin vessels are inactivated by immersing him in a bath at either 15° C. or 40–45° C.

In the chloralosed cat compensation by the skin vessels appears to be absent, as judged by absence of a reduction of limb volume or of electrical resistance of the skin, but this is not due to a loss of excitability of the cutaneous vessels due to anæsthesia since they react to adrenaline, to alkali and, reflexly, to sensory stimulation, as shown by the same methods.

In such an animal there must be, therefore, an absence of some stimulation which excites cutaneous vaso-constriction in man during over-ventilation.

The only possible stimuli not present in the chloralosed cat are those concerned with the active forced respirations, which it is suggested bring about constriction of the vessels of the skin in man during over-ventilation and maintain the blood-pressure.

The influence of ether anæsthesia upon the gaseous composition of blood. By N. E. Pitt. (*Preliminary Note.*)

The oxygen of arterial blood is found to be lowered about two volumes per cent. There is a rapid fall in the O_2 as soon as anæsthesia is induced and during the state there is some variation according to the depth of anæsthesia—when the rabbit is more deeply under the anæsthetic the O_2 falls still lower.

Exp. 10.

	Vols. per cent.		
	CO_2	O_2	N_2
Normal animal	47·0	16·6	1·6
Light anæsthesia	39·3	15·0	1·4
	42·2	14·9	1·6
Deep anæsthesia	44·2	14·2	1·5
	42·0	15·0	1·8
Recovery	47·9	16·1	1·4

The fall of O_2 content cannot be removed entirely when 60 p.c. of O_2 is given with the inspired vapour, but with 80 p.c. O_2 it entirely disappears.

Ether anæsthesia (after the effect of induction has passed off) causes a lowering of the CO_2 value, usually to the extent of 5 to 10 vols. per cent., and the fall varies inversely with the depth of anæsthesia, *i.e.* light anæsthesia is marked by a low CO_2 value and deep anæsthesia involves a high CO_2. In deep anæsthesia the CO_2 value may exceed the normal.

The high CO_2 values found by previous observers in cases of chloroform anæsthesia correspond to the deeper stages of ether anæsthesia.

The extent of the lowering of O_2 content is too great to be explained entirely as a result of shallow respiration, more especially when the occurrence of such low parallel CO_2 readings is taken into account. In view of this the rate of gas exchange of normal and anæsthetised blood was compared. (A constant volume of air was bubbled through a constant quantity of blood in 30 seconds and the blood gases were measured before and after.) These experiments demonstrated a decreased rate of absorption of O_2 (about one-third normal) and an increased rate of loss of CO_2 (about three times normal) in the anæsthetised blood.

Exp. 15.

	Vols. of gas per cent. in blood						O_2 gain	CO_2 loss
	CO_2	O_2	N_2	CO_2	O_2	N_2		
Normal	52·0	14·7	1·6 →	50·3	16·8	1·7	= 2·1	1·7
Etherised	41·0	14·5	1·6 →	34·4	15·2	1·6	= 0·7	6·6

Comparing plasma and blood, the rate of CO_2 loss is much greater in plasma, *i.e.* the cells normally exert a hindering effect on excessive CO_2 loss. Thus the effect of ether on this rate of CO_2 loss can be classified under the heading of "decreased activity of the cell."

Exp. 19.

Normal blood lost 1·5 vols. per cent. CO_2 by air treatment.
Its plasma lost 5·5 vols. per cent. CO_2 by air treatment.

The preparation of oxyhæmoglobin crystals from ox blood.

By Wilfrid Marshall.

A reliable method of preparing oxyhæmoglobin crystals in quantity from ox blood has not been described. Hoppe-Seyler's method is tedious and with slaughter-house blood, except that of the pig, is not satisfactory. Most of the irksome manipulations are unnecessary. It is desirable to work at as low a temperature as is convenient, but the

preliminary work may be carried out at room temperatures; the ether may be advantageously used in place of part of the alcohol to aid crystallisation; and the stay in the separator need not be unduly prolonged. The most objectionable feature of Hoppe-Seyler's method, however, is the use of undiluted alcohol. When added to an ox blood solution, however slowly, to one-third the volume recommended, it causes considerable decomposition of the hæmoglobin. The use of diluted alcohol greatly diminishes this change. The following method was found to give, on the whole, the best results.

Simplified Method. Centrifuge the ox blood. Remove the serum and wash the residual corpuscles three times with 1 p.c. salt solution. Measure the separated corpuscular mass and add an equal quantity of tap water; shake well. Place the mixture in a separator; add an excess of pure ether; shake and allow to stand overnight for separation to occur. Run off the bottom clear solution; add to this by rapid dropping during constant agitation, half its volume of a mixture of 70 parts commercial alcohol (96 p.c.) and 30 parts ether-saturated water. Allow to stand half an hour; filter. Put the filtrate into a refrigerator or vacuum-freezer at − 5° C. Crystals separate within 24 hours and a large crop is obtained in 2–3 days.

If time is pressing, the lower part of the blood mixture may be run off after about 2 hours' stay in the separator and filtered, and the diluted alcohol then added. The maximum concentration of alcohol which the mixture will tolerate without apparent change is 16 p.c. This strength, obtained by running slowly into the blood solution half its volume of 50 p.c. alcohol in ether-saturated water, however, gives only a small yield of crystals and occasionally produces slight turbidity on standing which interferes with crystallisation.

A further crop of crystals can be obtained from mother liquors by adding slight excess of pure ether and returning to the refrigerator.

The moist crystals at room temperatures, or slightly above, dissolve in little more than their own weight of water. Recrystallisation is effected by pouring off the supernatant fluid, washing the crystals rapidly with ice-cold ether-saturated water, melting the crystalline mass by the warmth of the hands or of tepid water, filtering to remove any trace of amorphous material, and replacing in the refrigerator. The second crop of crystals is collected on a porous tile and, after the excess of fluid has drained off, the crystals are dissolved in the smallest quantity of water and the solution, after filtering, is kept at a temperature of approximately 0° C. Freezing of the liquid must be avoided.

The crystals may be kept in a moist state in a closed vessel filled with

oxygen for considerable periods with but slight change to methæmoglobin. If air-dried, the crystals are converted into methæmoglobin but retain their form. Dried in a desiccator over sulphuric acid they are changed to a substance giving a brown solution with no characteristic absorption bands.

Benzene, chloroform, methyl, propyl, or other alcohols as adjuvants to, or substitutes for, ethyl alcohol offer no advantages.

Effect of drugs on protein content of cerebro-spinal fluids of rabbits. By L. F. Hewitt and H. Florey.

In the course of some other investigations it became desirable to know whether the permeability to proteins of the cerebral blood vessels could be altered by the administration of drugs.

The procedure adopted for the collection of the cerebro-spinal fluid was devised to avoid any possible blood contamination. The rabbit was rapidly killed by chloroform and its head then fixed in a flexed position so as to put the posterior atlanto-occipital membrane on the stretch. A longitudinal incision was made in the mid-line down to this latter structure and the muscles retracted so as to bare it. The membrane was thoroughly dried with cotton-wool. A small hole was then burnt through it by means of a red-hot needle. Through this hole the fluid—quite clear and blood free—was sucked into a dry glass pipette. This method of collection was found to yield about 1 c.c. of fluid.

The following drugs were used in an endeavour to increase the protein content—*para*phenylenediamine, urotropine, pilocarpine and histamine.

*Para*phenylenediamine gives a remarkable œdema of the head and neck which has been found to be due to the escape of whole plasma from the blood vessels concerned (Tainter and Hanzlik). It was thought that this drug might alter the cerebro-spinal capillaries in the same way.

Urotropine passes into the cerebro-spinal fluid and it is claimed by de Arric and Millet, who used a precipitin technique, that the permeability of the capillaries is also increased slightly. Pilocarpine is a "lymphogogue," and histamine increases the permeability of blood vessels in parts of the body.

The volume of the fluid was measured into a small centrifuge tube, 0·4 c.c. of 10 p.c. sodium tungstate and 0·5 c.c. of 8 p.c. sulphuric acid were added. The tube was centrifuged and the supernatant fluid decanted off. The precipitate was suspended in water, dissolved by adding one drop

of 20 p.c. sodium carbonate solution; 0·05 c.c. of a phenol reagent (Wu) and 0·5 c.c. of 20 p.c. sodium carbonate were added and the volume made up to 5 c.c. The colour produced was compared with that given by tyrosine with the same reagents, whence the protein content may be calculated.

Drug	Dose g. per kg.	Fluid taken after	No. of animals	Protein p.c.
None	—	—	4	0·040
p-Phenylenediamine	0·2 subcutis.	2½ hrs.	2	0·052
Urotropine	1·0 intraven.	1½ hrs.	1	0·040
Histamine	2·5 mg.	30 mins.	1	0·036
Pilocarpine	0·6 mg.	2½ hrs.	1	0·040

In no case was there an appreciable alteration in the protein content except in the case of *p*-phenylenediamine where a 25 p.c. increase was observed; but this apparent increase is negligible compared with that obtained in man in tubercular meningitis, *i.e.* usually about 400 p.c. *p*-Phenylenediamine evidently passed into the fluid since on standing a blue colour developed. The figures for normal rabbits are higher than those for human fluids.

Recovery of a pancreatic secretory excitant by vivi-dialysis of the circulating blood. By H. Necheles and R. K. S. Lim.

(*Peking Union Medical College.*)

Following the fundamental discovery of *secretin* by Bayliss and Starling (1902), others, viz. Enriquez and Hallion (1903), Fleig (1903, 1904), Matsuo (1912–3), have succeeded in demonstrating the existence of a pancreatic excitant in the circulating blood. The final proof, however, *i.e.* the identification of the circulating excitant with secretin, is still lacking. It is, of course, essential first to isolate the excitant from the blood. We wish to report the results of preliminary experiments in which we have endeavoured to recover the excitant from the circulation by vivi-dialysis.

Dogs were starved for 16–36 hours; the carotid-jugular or the femoral vessels were cannulated under local anæsthesia and connected with the dialysing cylinders[1]. Heparin was given as anticoagulant. After a control dialysis of 2 hours, the dog was fed or *N*/10 HCl was injected into the duodenum (in the latter case, a general anæsthetic was employed). The dialysate (Locke's solution) was then changed, and the experiment continued for another 2 hours. The dialysates were used unconcentrated

[1] Details of technique described by H. Necheles in *Chinese Journ. Physiol.* 1. 69–80. 1927.

or after concentration and precipitation of most of the salts with alcohol. The final substance, a yellow oily fluid, was tested for its pancreatic exciting property on normal dogs under ether.

Eight experiments were performed. Two of these were vitiated in that the blood pressure fell as the experiment proceeded, resulting in a slow circulation rate and some clotting in the dialysers. In both experiments, the control dialysate gave a positive result, while the dialysate obtained after feeding was less or equally potent.

The injection of the unconcentrated dialysate failed to show any pancreatic effect.

The remaining five experiments were apparently carried out under favourable conditions. Of these three (one *N*/10 HCl in duodenum) gave positive results, the control dialysate having little or no effect, while the dialysate after feeding produced a pancreatic response. One (*N*/10 HCl in duodenum) was completely negative, while another gave equally positive responses to both dialysates.

There is thus undoubtedly a pancreatic excitant in the circulation, which may be present before stimulation and which in three out of five satisfactory experiments was increased after stimulation. We were not able to state the occurrence of an increase after stimulation in the case of the gastric excitant (Lim and Necheles, 1926).

Since both gastric and pancreatic excitants were prepared in the same way, the possibility of their identity is not remote. The identity of our dialysed excitant with secretin obtained from intestinal extracts, remains to be investigated, although we have found that Luckhardt's method of salting out secretin from extracts, fails to precipitate the dialysed excitant.

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The effect of insulin on the blood sugar of the pig. By H. E. MAGEE and D. HARVEY, *The Rowett Institute, Aberdeen.*

Raper and Smith(1) found that when insulin was added to defibrinated cats' blood perfusing through the hind limbs of a cat the rate of disappearance of glucose was much accelerated, thus confirming the findings of Burn and Dale on the eviscerated animal. When, however, pigs' blood was perfused the characteristic effect of insulin was not obtained although the removal of sugar by the muscles in the absence of insulin was not affected in any way. Prof. Raper, discussing these results with one of us (H. E. M.) recently, suggested the possible existence of an anti-insulin substance in pigs' blood. Accordingly we did some experiments on the effect of insulin on this animal's blood sugar. Sugar was determined in triplicate by the Hagedon-Jensen method. Two pigs were used but as the results were similar in both the findings in one only are given. The pigs had not received food for 15 hours before injection:

1. 8. 27. Pig 19·6 kg. 7 units insulin (subcutaneously).

Mins. after injection	Sugar mg. per 100 c.c. blood	*p*H	Remarks
0	79	—	Animal became very dull. No convulsions.
20	65	—	
40	68	—	
60	59	—	
80	65	—	

3. 8. 27. 18 units insulin at 9.50.

0	108	—	Severe convulsions at 11.55. Recovery commencing at 12.10. Recovery accelerated by intramuscular injections of glucose at 12.22.
30	93	—	
60	74	—	
90	77	—	
120	75	—	
150	66	—	

11. 8. 27. 20 units insulin at 10.10.

0	110	7·30	Very severe convulsions at 11.50 which abated but returned at 12.3. Complete recovery at 7.30 p.m. after repeated injections of glucose and adrenalin.
30	75	7·33	
60	84	7·30	
90	75	7·30	
20	65	—	

These results suffice to show that the pigs' blood sugar responds much as that of other animals to insulin injections. The pig appears to be much more sensitive to hypoglycæmia than the usual laboratory animals; for in the second and third experiments convulsions began when the blood sugar was about 75 mg. per 100 c.c. In man it is about this level that premonitory symptoms of hypoglycæmia begin to appear so that the pig would appear to be even more sensitive to hypoglycæmia than man.

In the third experiment the *p*H was determined. Apart from a small increase 30 minutes after insulin it remained unchanged. The only other experiments in this connection we know of are by Brugsch and Horsters(2), who found that insulin lowered the *p*H of fasting rabbits but had no effect after food.

REFERENCES.

1. Raper and Smith, Journ. Physiol. 62. p. 17. 1926.
2. Brugsch and Horsters, Biochem. Zeit. 175. p. 130. 1926.

The active relaxation of capillaries and venules in "reflex flare." By August Krogh and P. Brandt-Rehberg.

(*Laboratory of Zoophysiology, Copenhagen.*)

In Lewis' book on the blood vessels of the human skin which has greatly increased our understanding of capillary reactions there is a point on which Lewis himself seems to be in some doubt, and which is certainly of minor importance to his main argument, while, from our point of view, it concerns the very foundations of our conceptions regarding arteriolar and capillary control of the blood supply.

Lewis concludes from his experiments given in detail on pp. 41–45 that in the vividly red zone or "flare" surrounding a stimulated spot the "strong arterioles" are actively relaxed by an axon reflex in sensory fibres, while the "minute vessels," comprising smaller arterioles, capillaries and venules, are passively expanded by the resulting pressure. According to our views a purely arteriolar dilatation can only produce an increase in flow which may or may not cause a *slight* increase in the blood colour of the skin, at the same time as it raises the skin temperature to a few tenths below that of the arterial blood, while vivid colour reactions like the flare must necessarily involve an active relaxation of the capillaries and especially of the venules.

A discussion of all pertinent arguments will be given in the forthcoming new edition of Krogh's *Anatomy and Physiology of Capillaries*, but in view of the importance of the point we could not rest content with arguments, but have attempted to devise a crucial test. We have found it possible to obtain an approximate measure of the pressure in the venules by applying a transparent Recklinghausen capsule connected with a manometer and noting the pressure necessary to produce a distinct blanching. When applied to a yielding surface this method will give inconstant and much too high results, because the pressure is mainly taken up by the elastic tissue; but in skin which is directly supported by bone, as on the knuckles or the bent elbow, quite definite readings can be obtained. Even these are probably slightly too high, but they are sufficiently accurate for our purpose.

When determinations are made at the height of the clavicle, so as to exclude any static pressure, we find in vivid histamine flares pressures varying from 6 to 11 cm. of water. Miss Carrier found pressures in normal nail fold capillaries of 4 to 8 cm. water, and the doubtful increase observed by us is certainly insufficient to cause any perceptible expansion, since the pressure will rise much higher when the arm is brought down from the elevated position. We conclude, therefore, that in a histamine flare the dilated venules must be actively relaxed just as are the arterioles.

Petroleum emulsion in the small intestine. By JOHN MELLANBY.

Emulsified fat, containing bile only, is readily absorbed from the small intestine, and passes into the mesenteric lacteals in a finely divided condition(1). Therefore the presence of bile, and not hydrolysis by lipase, is necessary for the absorption of fat by the villi of the small intestine. During absorption there is a large secretion of pancreatic juice and an increased secretion of bile. These secretory effects are due to the absorbed bile salts carrying secretin from the cells of the intestinal mucosa into the portal blood(2).

Experiments were carried out to determine whether fat absorption depends upon the physical and chemical properties of fat, or whether emulsification and the presence of bile salts are the essential factors. The problem was investigated by means of liquid petroleum, which consists of chemically inactive paraffins, and forms a stable emulsion when shaken up with bile.

Cats, anæsthetised with veronal, in which cannulæ had been tied into the pancreatic duct, the gall bladder and the bile duct, were used as the experimental animals. Liquid petroleum (5 c.c.), emulsified by shaking with cat's bile (1 c.c.), was injected into the duodenum. Examination of the intestine and mesentery during a period of six hours showed that there was no absorption of petroleum into the lymphatic system of the intestine although the oil passed rapidly down the intestine.

During the experimental period there was only a very small secretion of pancreatic juice (two drops) and no augmentation of the spontaneous secretion of bile—observations which indicate that the oil had prevented the absorption of bile salts by the intestinal mucosa.

The results indicate that during the passage of emulsified petroleum down the intestine, the processes of absorption are stopped. It is evident, therefore, that the chemical and physical properties of fats, in addition to the presence of bile salts, determine the absorption of fat through the intestinal mucosa into the lacteals.

It is interesting to observe that liquid petroleum inhibits the absorption of bile from the intestine and therefore prevents the secretion of pancreatic juice and an increase in the secretion of bile. In this way liquid petroleum stops digestion and absorption in the small intestine, and thereby diminishes the nutritive value of any food with which it may be admixed.

1. Mellanby. Proc. Physiol. Soc., Journ. Physiol. 64. 5. 1927
2. Mellanby. Journ. Physiol. 61. 419. 1926.

PROCEEDINGS
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The electrical responses from a strip of curarised skeletal muscle under various conditions. By W. H. Craib[1]. (*University College Hospital Medical School.*) (*Preliminary communication.*)

Method. A decerebrate frog was curarised by injecting 2 per cent. curare into the dorsal lymph sac. The sartorius was removed with a minimum of injury by dividing the tibial tendon, the connective tissue adhesions between it and underlying muscles, and its muscular pelvic attachment. Threads were attached to pelvic end and tibial tendon. It was suspended between two upright supports at a tension sufficient to render contraction isometric. It was stimulated at the pelvic end by parallel electrodes so placed that the long axis of the tissue was perpendicular to the plane passing through the electrodes. Thus suspended the tissue is surrounded by only a very limited layer of conducting (body) fluid. To vary the quantity of the surrounding conducting fluid a flat circular glass dish with perpendicular walls 5 mm. high was placed on a movable support so that it could be gently raised beneath the suspended tissue until any required length of the latter was in contact with the surface of saline filling the dish. At least 1 cm. of muscle from the pelvic (stimulated) end was left surrounded by air in order to diminish the effect of the artificial stimulus on leading off contacts placed on the tissue in the dish. When studying the effects of injury on the action current response, before suspending the tissue as above, the tibial end of the muscle was dipped for a few seconds into saline heated to 60° C. Leading off contacts were non-polarisable and pointed to a diameter of $\frac{1}{2}$ mm. Responses were recorded by a string galvanometer with a string sufficiently tense to secure accuracy of deflection. Elements of the artificial stimulus were satisfactorily excluded from action current responses by (1) killing the tissue *in situ* and examining the movement of the string during stimulation; or (2) reversing the direction of the stimulating current and observing its effects on the recorded response; or (3) photographing a simultaneous record of stimulus and action current response by means of a second string. To simplify interpretation of records, the tissues were examined at a temperature of about 12° C.

[1] Work undertaken on behalf of the Medical Research Council.

Results. (*a*) With two contacts on a sartorius suspended in air the response is diphasic, the initial phase indicating a relative negativity of the proximal contact.

(*b*) With two contacts on a sartorius in contact with a quantity of saline, the response varies in form with the distance between the contacts. It shows from three to six phases. The initial deflection invariably indicates a relative positivity of the proximal contact.

(*c*) With one contact on a sartorius immersed in a quantity of saline, and another at a distance in the surrounding medium, the response is triphasic with an initial positive phase.

(*d*) With the distal contact on an injured area, and the tissue suspended in air, the response is monophasic. If, without disturbing the relation between tissue and leading off contacts, the regions led off are placed in contact with a quantity of saline, the response is no longer monophasic and shows an initial positive phase.

Conclusions. Diphasic and monophasic responses may be obtained from a curarised sartorius under particular conditions only, *i.e.* when the tissue is surrounded by a very limited conducting medium. They are not obtained when the tissue is surrounded by a large quantity of saline. Granted that these are the facts, prevailing views on the explanation of the electrical responses of skeletal muscle appear to be called in question.

The active relaxation of capillaries and venules in the reflex flare. By T. Lewis.

In writing of the reflex flare produced in human skin by histamine, etc., I have recently expressed the view that this flare is due to the opening up of the strong (strongly muscled) arterioles, and have concluded that, so far as the minute skin vessels are concerned, any active participation in this reflex is inappreciable. In stating that an active change in the minute vessels can play no more than a minor part in the flare, I have intentionally inferred that the dilatation of these vessels, which is responsible for the visible redness of the skin, is brought about mainly, perhaps entirely, as a passive effect (*The Blood Vessels of the Human Skin*, London, 1927, pp. 42 and 221). With this view Krogh and Rehberg express disagreement in *Proc. Physiol. Soc.*, Nov. 12th. They have attempted to ascertain the pressure prevailing in the minute venules of skin involved by vivid histamine flares, using a capsule similar

to that of Recklinghausen; and they declare the pressure to be from 6 to 11 cm. of water. Since they find that such pressures are little if at all greater than those existing in the same vessels when these are unflushed, Krogh and Rehberg conclude that in the histamine flare the dilated venules must be actively relaxed. If Krogh and Rehberg's values for venule pressures are correct, then the conclusion that these vessels are actively dilated in the histamine flare seems to me inevitable. However, I am unable to accept their values either as correct or as even approximately representing the true values. They believe that the pressure applied externally by means of a transparent capsule is equal to and gauges that in the venules when "a distinct blanching" of the skin occurs. Their use of this index is eventually the result of two assumptions, first, that the index correctly gauges venule pressure in the normal skin, and, secondly, allowing this to be the case, that an identical index serves when the skin is flushed. Upon the first assumption no further comment is here needed; the second assumption can be shown to be erroneous in the following way. If a known pressure (say 40 mm. Hg) is thrown upon the veins of the arm, it is clear that, after a sufficient lapse of time, the pressure in the minute venules will rise to at least that value. If now, a pressure exactly equal to this congesting pressure, is thrown upon the congested skin by means of a capsule, this skin at once blanches; it blanches, not only "distinctly," but conspicuously; "distinct" blanching is obtained by smaller external pressures, pressures that are known from the circumstances of the experiment to be much below the true values in the venules. The amount of actual blanching seen, when equal congesting and testing pressures are used, increases progressively as these pressures are raised in successive tests. In all such tests the congested skin blanches to approximately the same *end point*, namely, to its original uncongested colour (tested over the range 20 to 70 mm. Hg). Thus it is abundantly clear that such an index as "distinct blanching" is inadmissible in the case of skin flushed by congestion. A truer index, and one which gives a value for the pressure within the minute vessels that cannot exceed the actual value, is the pressure which, applied externally to the congested skin, reduces its colour to that of uncongested skin. If this index is used in the case of histamine flares upon skin placed level with the base of the heart, values of 50 to 60 mm. Hg are obtained[1]. These are minimal values for the actual pressure in

[1] If the index is "distinct blanching" then I agree that the values are very much lower, but that is also so for the skin when the pressure in the minute vessels is raised by congesting the veins to 50 or more mm. Hg.

the minute vessels, and they strongly support my idea that these vessels are dilated passively and not actively as Krogh and Rehberg believe.

A full account upon which this preliminary note is based will appear in *Heart*.

The effects of subcutaneous and intraperitoneal injection of oxygen upon the oxygen saturation of the arterial blood.

By H. W. Davies and M. Rabinovich.

Dogs anæsthetised with luminal sodium were rendered anoxæmic by the production of pulmonary embolism in the manner described by Binger, Brow, and Branch[1]. The anoxæmia was associated with considerable increase in respiratory rate which returned to normal or fell considerably when oxygen was administered by the lungs. Oxygen administration by this method restored the oxygen saturation of the arterial blood to normal whereas massive injections of oxygen subcutaneously and intraperitoneally were without effect upon the respiratory rate and were associated with little or no increase in the oxygen saturation of the arterial blood. A summary of these effects is given in the table. It may be concluded that the use of subcutaneous and intraperitoneal injections of oxygen for the treatment of anoxæmia cannot be justified.

Table I.

Dog No.	Weight kgm.	Normal. % arterial oxygen saturation	Anoxæmic. % arterial oxygen saturation	After subcutaneous O_2 injection, % arterial oxygen saturation	C.c. O_2 injected subcutaneously	After intraperitoneal O_2 injection, % arterial oxygen saturation	C.c. O_2 injected intraperitoneally	Breathing oxygen, % arterial oxygen saturation
I.	14	92	85	87	150	86	1000	—
II.	23	89	81	81	150	83	1000	94
III.	13	88	78	87	200	—	—	97
V.	19	87	80	85	350	82	500	95
VI.	17	86	56	43·5	300	—	—	94.5
VIII.	15	85	80	80	1000	—	—	98
X.	13	91·5	56	53	500	53	500	94·5

[1] *Journal of Clinical Investigation*, I. p. 155. 1925.